Overview and Integration of Cellular Metabolism

Prof. Arindam Ghosh

Dr. B.C. Roy Multi-Speciality Medical Research Centre

Indian Institute of Technology Kharagpur

Week 07

Lecture 32: Transformation of Amino acids

Hello everyone, welcome back to the lecture session on Overview and Integration of Cellular Metabolism. We are in week 7 and we are continuing with protein metabolism. Today's topic is how amino acids are transformed in various metabolic reactions. So basically what we will be discussing is the concept of deamination. We will be discussing the transamination reaction, what is the clinical significance. We will be discussing how some amino acids are decarboxylated and what is the concept of transdeamination, alright.

So first to understand how the amino acid pool is generated in the body, it is generated by various sources. The first one is the dietary proteins, alright. So dietary proteins are broken down in the stomach, in the small intestine. You have already gone through that class in digestion absorption of proteins, right.

From there the amino acids that are generated are thrown into the bloodstream. I say that I am thrown as if it is delivered, ok. It is delivered to the amino acid pool. So pool means, you know swimming pool where there is a lot of water. Similarly imagine a big tank of amino acids and the first source of amino acid is the proteolysis, breakdown of protein from the dietary sources.

Next endocytic pathway. What is endocytic pathway? It is the pathway where the cells engulf something, you know via phagocytosis, pinocytosis those are the phenomena where the particle goes inside the cell, it is degraded in the lysosomes and then they are delivered back to the surface. That is another pathway of proteolysis and that also contributes to the amino acid pool that breaks down, breakdown happens in the lysosomes, alright. So that is second source that is endocytic pathway. And the third source is again controlled proteolysis that happens in case of some tagged proteins, though tagged proteins are tagged using the ligand ubiquitin and after that tagging the intracellular proteolysis takes place in the proteosomes and that also contributes to the amino acid pool.

So basically these are the methods of proteolysis and ultimately when proteins are degraded the simpler form from all catabolic sources, I mean for all catabolic processes the big proteins are broken down into amino acid, but the catabolism does not end there. Amino acids once they are formed for considering all amino acids as you must be knowing it has this basic structure where there is a central carbon atom, there is an amino group and there is an carboxyl group, there is a side chain, alright and there is a hydrogen atom. So every amino acid is then again further broken down and that is the series of lectures that we will be having for the next few sessions that is how amino acid is catabolized and ultimately how the amino group is removed from the amino acid and what is the fate of this carbon skeleton. So this is the rest of the carbon skeleton. So this is the amine group that is common to all amino acid and depending on this are the side chain where the structure of all amino acid will vary.

So our first and foremost focus will be to remove the amine group or amino group. If you remove this we are only left with the carbon skeleton and then how the carbon skeleton is moved is actually varying from amino acid to amino acid and we will be studying that in lot detail in individual amino acid metabolism classes. But for the first the common theme is removal of amine group and it is same for all amino acids. So let us explore how it happens. So generally that happens in three broad ways.

Number one deamination, number two transamination and number three decarboxylation right. And what are the areas in which the I mean what is the organ where the amino acids are degraded the main site is the liver ok. So multiple choice question site of amino acid degradation if liver is an option you should always choose that option right. Now let us go into detail of each and every one of these reactions. So we will start with deamination.

If you just break the word deamination you will get the idea what does it mean. It means deamination removal of amino group. So that is the definition of deamination. Elimination of amino group from whom amino acid. How what will happen if the NH2 group is removed it leads to the formation of a very important metabolite that is ammonia.

So deamination are the common set of reactions which involves removal of the nitrogenous part that is amine group from amino acid in the form of ammonia. So in every deamination reaction NH3 or free ammonia will be generated. What is the fate of this ammonia? Again we will be studying in detail in the next class where we will be exploring how the where from the ammonia is produced what are the sources of ammonia how how it is degraded everything will be going to detail. So for now we

should know that one of the sources of ammonia is this deamination reaction. Now again if we classify the type of deamination reaction it is again of four types.

The most important being the first one that is oxidative deamination. It is the most important for human beings and all higher animals. That being said there are also few minor variation of deamination reaction. For example reductive deamination, hydrolytic deamination, intramolecular deamination. Let us cover this minor deamination reaction in the first slide.

So you can see whether an amino acid is reduced to a fatty acid or whether there is hydrolytic cleavage of an amino acid to a hydroxy acid or whether there is some intramolecular deamination that leads to formation of an unsaturated fatty acid. The common theme to all of them is production of NH3 or ammonia alright. Considering these example histidine is acted upon by histidase to form urocanic acid cysteine and serine. Cysteine is actually simultaneously deaminated and sulfide desulfurated by the enzyme desulfirase. It forms amino acid and ultimately by hydrolytic cleavage they form pyruvic acid.

So all of these are examples of minor pathways of deamination means these are the exceptions where ammonia are produced from multiple or specific sources. But what is the what is not the exception what is common to every reaction that is oxidative deamination. So this again if you are to choose which is the major form of oxidative deamination that releases ammonia the answer which is the major form of deamination excuse me that is oxidative deamination. So what is the enzyme that is the key player in oxidative deamination or oxidative deamination means the action of this enzyme glutamate dehydrogenase alright. Why this capital L again I told you in the previous class what do you mean by capital D and capital L depending on the configuration of the H and OH group of the penultimate carbon of CH2OH you already know that.

If you have missed that part it is up to you to find and let yourself know what do we mean by capital D and capital L isomerism it has been discussed right anyway. So in most organisms glutamate is the amino acid that serves as the final pathway by which this oxidative deamination takes place alright. In liver mitochondria there is an enzyme that is glutamate dehydrogenase that is catalysing this reaction. So what is the reaction? So it is acting upon glutamate it is being converted to an intermediate this is immunoglutamate right or often we say immunoglutarate because it is the intermediate form between glutamate and alpha ketoglutarate. So this intermediate form that is the shifts base is then spontaneously converted to alpha ketoglutarate.

So if we just look at the reaction if we just eliminate the unstable intermediate the

reaction looks like this glutamate is converted to alpha ketoglutarate by the action of water and NAD or NADP plus and in turn we get a reduced NAD or NADP plus and generation of ammonia right. So since water is coming it is actually reacting with NH3 plus to produce ammonium ion because NH3 is highly soluble right. So ammonia being highly soluble in water it reacts with water to form ammonium ion. So this is the glutamate dehydrogenase reaction GDH which converts glutamate to alpha ketoglutarate which requires NAD or NADP it produces NADPH or NADH and it happens in the mitochondria of liver ok and there is an intermediate shifts base production and most importantly it leads to the production of ammonia. So this is oxidative deamination.

So why why do you say so it is getting oxidized because there is removal of hydrogen who is taking up the hydrogen NAD or NADP plus right. So removal of hydrogen oxidation deamination so oxidative deamination the concept should be absolutely clear. So now a few things about this glutamate dehydrogenase enzyme it is a metalloenzyme very important it needs zinc to work with very important multiple choice question as a cofactor right it has got a zinc in its core. It is the only enzyme that can accept reducing equivalent in the form of NAD plus or NADP plus in other biochemical reaction metabolic pathway you need to be very careful whether it is NADH or NADPH whether it is NAD or NADP for example in glycolysis we are using NAD NADH for NHM patient you are using NADP NADPH fatty acids in this NADPH glyco generalizes again NADH. So be very careful except in this case you can either write NAD or NADP both will act, but please do not make this mistake where you are writing NAD plus and producing NADPH that does not hold good right.

So it should be NAD to form NADH or NADP producing NADPH right. Next this glutamate dehydrogenase enzyme has got 6 identical subunit can you tell me from the very second class what type of a enzyme has got multiple subunit right you are right that is allosteric enzyme. So needless to say this glutamate dehydrogenase enzyme has got allosteric regulators in the form of activators or inhibitors. So GTP and ATP are the allosteric inhibitors and GDP and ADP are the allosteric activators ok. Also steroid and thyroid hormone inhibit glutamate dehydrogenase multiple choice type of question you should be very mindful about that.

Next the low level of energy that this when the energy is low right body needs to catabolize amino acid to produce more and more energy that is when there is more and more TCA cycle right and then when there is more and more TCA cycle there is alpha keto that generates alpha ketoglutarate as a substrate for TCA cycle. So how now we know alpha ketoglutarate being a substrate for TCA cycle we all know as a part of anaplerotic reaction we need to replenish the intermediates of TCA cycle and this is one reaction where alpha ketoglutarate can be produced ok. Now regarding the metabolic

significance of glutamate dehydrogenase type of glutamate dehydrogenase reaction looking at the metabolic standpoint it is it can behave as both anabolic and catabolic. Since the reaction is reversible you see when we are considering catabolism it means the reaction is proceeding in such way so that the nitrogen are being removed from the body and ultimately it will be channeled to urea and when we are considering the anabolic fate it means the amination of alpha ketoglutarate by ammonia to form glutamate. So this glutamate dehydrogenase reaction can form glutamate that is the anabolic part because ultimately glutamate will be used to produce all other type of intermediates in body or all other essential compounds and whether when we are concerned with removal catabolism degradation again the same reaction oxidative deamination with the help of glutamate dehydrogenase will be happening and the reaction will be proceeding towards urea formation that is removal of ammonia alright.

So that was all about deamination. The next reaction is transamination ok. So transamination basically deals with transfer of amino group. Mind it in deamination reaction whether it was main deamination that is oxidative deamination or minor deamination there was always production of NH3 or production of ammonia. In this transamination reaction ammonia directly is not produced.

However, the amine group is transferred from one amino acid to another amino acid. Just see how it happens. Well the question may have been in your mind fine glutamate undergoes dehydrogenase to liberate ammonia, but where from we are getting glutamate right. So there this transamination reaction will answer your question. This is the second piece of the puzzle that fits in the amino acid catabolism.

So what is the basic principle of transamination reaction? So transamination so the enzyme is transaminase also known as aminotransferase. So if you hear this name aminotransferase it means one and the same thing ok. So basic principle is one alpha amino acid will be converted to one other amino acid and one alpha keto acid will be converted to other alpha keto acid. So basically if you see one amino acid and one keto acid is reacting together in a reversible reaction to produce another variety of amino acid and keto acid. So amino acid 1 plus keto acid 1 is forming amino acid 2 plus keto acid 2.

So one form of amino acid is getting converted to another form of amino acid by shifting of amine group from the amino acid to keto acid. So this ok now giving specific example to learn specific examples aminotransferases these are few specific example. Alanine aminotransferase or aspartate aminotransferase they are abbreviated as ALT and AST alright. So what is what they are doing? The reaction is alanine plus alpha ketoglutarate is forming pyruvate and glutamate ok. In case of AST what is happening aspartate and alpha ketoglutarate are reacting.

So this is the amino acid, this is the keto acid, this is the keto acid 2 and this is amino acid 2. Same in this case aspartate and alpha ketoglutarate react to produce oxaloacetate and glutamate. Now you see the common theme is alpha ketoglutarate is collecting the amino group and it is getting converted to glutamate. So glutamate is actually the common amino acid that is formed by transamination in these examples right. And generally the amine group is collected by alpha ketoglutarate, the amine group is transferred to alpha ketoglutarate that is the common keto acid ok.

So what is happening over here? Once the glutamate is formed now you already know what is the fate of glutamate. Earlier we studied right by oxidative deamination it requires an NAD or NADP plus. Now we can degrade this glutamate. What is glutamate getting converted to? It is getting converted to alpha ketoglutarate. So this alpha ketoglutarate can now be regenerated and takes place in another transamination reaction ok.

So one thing we should know that earlier this ALT or was I mean termed by the name serum glutamate pyruvate transaminase or SGPT. And this AST was also known as serum glutamate oxaloacetate transaminase or SGOT. Since there is production of pyruvate and glutamate it was named like that. Since this AST is producing glutamate and oxaloacetate it is named as that. You may still find it in many text books or many clinicians or physicians they are using this term SGOT SGPT.

Mind it SGPT means ALT and S GOT means AST alright. Now what is the mechanism of transamination? Knowing this reaction is fine, but we need to understand in more detail right. So first and foremost this transaminase enzyme requires a coenzyme it needs vitamin B6 that is pyridoxine. And what is the active form of the coenzyme? It is pyridoxal phosphate. So this is the structure of pyridoxine and this is the structure of pyridoxal phosphate which is abbreviated as PLP.

So if we just look I mean if we consider any reaction where a compound A and B reversibly forms compound C plus D generally what happens A and B will react to form an unstable intermediate that is AB ultimately it will get internally converted and ultimately this will dissociate to compound C and D right. Major reaction or almost all type of reaction happens in this way where two reactants combine to form an intermediate product and ultimately those unstable intermediate dissociate to form substrates or I mean products right. In this case it is not so. So what happens this coenzyme actually takes place in the I mean takes part in the reaction. So in first step what happens the amino group of the amino acid is transferred to pyridoxal phosphate ok.

It forms pyridoxamine phosphate and in the first step only the keto acid is released. And it is in the second step where alpha ketoglutarate reacts with the pyridoxamine phosphate to form glutamate and the pyridoxal phosphate is regenerated. So it is not that the amine group is directly transferred to the alpha ketoglutarate and it is released into formation of two products no. So let us see again how it happens. So this pyridoxal phosphate first accepts the amino group to form an pyridoxamine phosphate.

This is the intermediate form of the coenzyme or amino form of the coenzyme. And in the first step one of the product is released. In the next step the keto acid is reacting with the amino form of the coenzyme in order to produce the second amino acid and ultimately the active form of the enzyme is regenerated and it can again take part in another transamination reaction. So if we look at the overall reaction it appears as aspartate plus alpha ketoglutarate is forming oxaloacetate and glutamate. But in reality what is happening? The enzyme is being converted to enzyme intermediate.

First substrate is coming and first product is already generated and then second substrate is coming and then the second product is generated. Generally I told you what happens? Two substrates comes and two products are generated simultaneously. But not in this case over here since the reaction mechanism happens in this to and fro way it appears as if a ping pong ball is bouncing on a table. Ping pong Chinese is also known as table tennis in Indian games right. So it is because of this phenomena due to which this transamination reaction is an example of a ping pong mechanism ok.

Ping pong mechanism of reaction or a bisubstrate reaction or one substrate is getting converted to one product and then next substrate is getting converted to next product. So what is the clinical significance of transamination? So these two enzymes that we discussed AST and ALT actually are induced by glucocorticoid which flavor gluconeogenesis. This is the metabolic fact and regarding the clinical importance the value of AST if it is increased it can give us an indirect idea about a clinical condition that is myocardial infarction. This is a very important diagnostic enzyme. So if AST is raised one of the major reasons we can think of is myocardial infarction.

And whenever the value of ALT alanine transaminase is raised we generally think of some error with the liver metabolism or hepatic metabolism. In liver diseases the level of ALT will be increased. Of course thereafter we need to corroborate with other clinical history other findings but this is very one important marker that will give us an idea about organ disorders. So this is an important diagnostic enzymes. Now a very important multiple choice type of question that is what amino acid do not undergo transamination reaction? So what are the amino acid that does not takes place all amino acid undergoes

transamination except these four lysine, threonine, proline and hydroxyproline they do not undergo transamination reaction.

They have got their own dedicated enzymes by which the amine group will be removed from them. So what is the importance of transamination reaction? Well this is the first step of catabolism right I told you the ammonia amine group is removed and ultimately it we are left with the carbon skeleton and then the carbon skeleton enters into other catabolic pathways which will be again dealing which is different for different amino acids there will be studying them in lot of details. But for this class we should know that the transamination is actually helping in synthesis of many non-essential amino acids. For example pyruvate can be transaminated to alanine oxaloacetate produces aspartic acid alpha ketoglutarate produces glutamic acid right. So many amino acids can be synthesized in the body in this way.

So we do not need those amino acids to be consumed right because our body can produce them. There are few exceptions I mean some amino acids that cannot be produced in this manner those should be provided in the food and those amino acids are known as essential amino acids. The amino acid that can be formed using transamination are non-essential amino acids. So how actually it can happen? For example not only it helps in synthesis of the non-essential amino acid it can also act in an interconversion pathway. For example suppose both of these amino acids are non-essential amino acid the amount of amino acid 1 is very high and the amount of amino acid 2 is very low.

So what will amino acid 1 do? It will simply takes take part in the reversible reaction where the amine group will be transferred to a keto acid and to lead to production of amino acid 2. So the level of amino acid will be balanced depending on the requirement. This is the chart that shows what are the essential and non-essential amino acid. What is semi-essential amino acid? They can be produced in the body but sometimes the requirement is often hampered in case of special situations. For example, cytosine can be synthesized from phenylalanine, cysteine can be synthesized from ethionine we will be going into details when we are dealing with this individual amino acid metabolisms right.

So what is trans deamination? Trans deamination is basically coupling of the whole thing. So transamination of any amino acid leading to the formation of glutamate and ultimately by the action of glutamate dehydrogenase oxidative deamination we are producing alpha ketoglutarate and ammonia. So transamination actually takes place in cytoplasm of all the cells whereas, we already read oxidative deamination only takes place in mitochondria and liver because only liver contains glutamate dehydrogenase. So basically two phenomena which are located metabolically apart are actually

physiologically coupled together.

Hence this is known as trans deamination. Now we should know that generally amino acids are deaminated at the rate of 50 to 70 gram per deciliter and I already told you that it can accept NAD or NADP plus as a coenzyme. So NAD and NADP plus. Now one thing to note this hydrolysis of glutamine also yields ammonia right, but this is mainly happening in the kidney and we will be discussing this in detail when we are discussing in the metabolism of ammonia in the next class. So we are left with another mechanism that is decarboxylation. So where the carboxyl group is actually the removed right and this is another way where the I mean the amino acids are catabolized right.

This is not the removal of ammonia right. So we are forming amines. This amines can now be acted upon by various amine oxidases to produce ammonia, but few amino acids when you are discussing amino acid catabolism decarboxylases should not be omitted because few very important compounds are formed using decarboxylase enzymes. For example, glutamate is decarboxylated to gamma amino butyric acid is a very important neurotransmitter and histidine is converted to histamine which is very important neurotransmitter or mediator of allergic reaction. Now we discussed in brief that what are the fates of the carbon skeleton and this diagram will again be discussed as we discussed each and every individual amino acid. So depending on the metabolic fate of amino acid the carbon skeleton of all 20 amino acids are actually fueled into 7 major metabolic intermediates. So either they can enter into carbohydrate metabolism those are known as glucogenic amino acids that are in pink shade or they can produce ketone body right and there are some amino acids they can form both glucose and ketone bodies right.

But we do not need to stress much for now because we will be discussing each and every amino acids and we can easily tie them in the integration of metabolism right. So this is the fate of amino acids how amino acids are produced in the body and this is ultimately how they are degraded we just discuss this part and we will be discussing this part in future alright. So this is the conclusion in this class we have covered what is the mechanism of deamination what are the types of deamination what is the mechanism of transamination what is the clinical significance of transamination how many or how the amino acids are decarboxylated what is the feature of trans deamination and we have briefly touched what are the metabolic fate of all amino acids. So these are my references and I thank you all for your patient hearing.