Medical Committee
The University of Jordan

SLIDE SHEET

LECTURE#: 24

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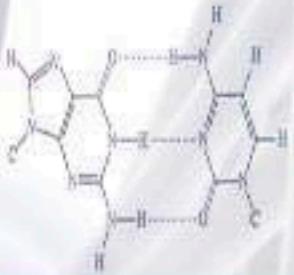
Biochemistry



Majida Al-Foqaraa'

Catabolism of The Amino Group

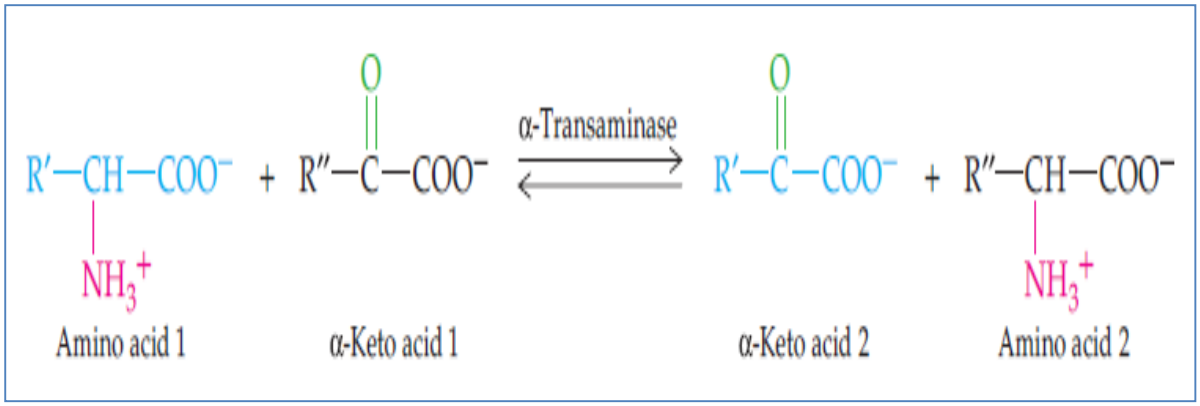
Let's continue on with what we discussed yesterday; the general scheme of amino acid metabolism and how proteins will be degraded to their corresponding amino acids. The amino acids' amino groups will be removed to make nitrogen-containing compounds, and the carbon skeleton is used to make glycolytic intermediates and intermediates for citric acid cycle.

How do we remove the amino group from amino acids?

We discussed this topic during our summer semester. What we mentioned was the conversion of amino acids to keto acids. This process is called **transamination**. We discussed the conversion of amino acids to keto acids in the case of PLP (pyridoxal phosphate), a derivative of Vitamin B6.

What is the function of transaminases?

To convert amino acids into keto acids. When the amino group is removed from any amino acid, it forms a keto acid, and when an amino group is added to a keto acid, it will form an amino acid. This is the difference between them.



There are 3 amino acids and their corresponding keto acids that you are required to memorize. They are the same ones you were required to memorize during the summer semester.

Those amino acids and their corresponding keto acids are:

1)**Aspartate to Oxaloacetate**: when you remove the amino group from aspartate you'll have oxaloacetate. Oxaloacetate and Aspartate both have 4 carbons because **transamination does NOT change the number of carbons.**

2)**α-ketoglutarate and glutamate:** α-ketoglutarate has 5 carbons because it is the product of the decarboxylation of iso-citrate, therefore, you can assume glutamate also has 5 carbons.

3)**Alanine and Pyruvate:** Which are both 3-carbon compounds

* Enzymes that transfer the amino group are called Transaminases or they can be called aminotransferases.

We have many transaminases. Each transaminase is either specific to one amino acid, or to multiple amino acids. It will remove the amino group of an amino acid and transfer it to a keto acid.

Which keto acid will it be transferred to?

Most transaminases are specific for α-ketoglutarate. Within the active site, an amino acid and keto acid should be included. Most of the time the keto acid is α-ketoglutarate.

So what should also be a product of most transaminations (if you assume the reversible reaction has occurred)?

Glutamate

Transaminases are present in equilibrium. Their equilibrium constant is almost 1. That means that at equilibrium, the concentration of the reactants is equal to the concentration of the products. This means that this reaction is reversible, and the increase in concentration at one side, either the reactants or products, will shift the reaction in either direction. That means that this reaction can be used for both the synthesis and degradation of the amino acid that we are dealing with.

Examples: Alanine can become pyruvate, if we have too much Alanine the reversible reaction will favor the production of pyruvate. If we have a lot of pyruvate, it means we also have a lot of energy, so we need to convert the unnecessary molecules to amino acids. And if we have a mess of amino acids, we should convert them to energy molecules. The same applies to α-ketoglutarate; an intermediate in the citric acid cycle,

if we have a high amount of it, we convert it to amino acids. If we have it in low amounts then amino acids will give you energy, instead.

All aminotransferases require the coenzyme pyridoxal phosphate, for them to work.

Why do we like to talk about transaminases?

We have two important transamination enzymes **AST(Aspartate transaminase)**, which converts aspartate to oxaloacetate and **ALT(Alanine transaminase)**, which converts alanine to pyruvate.

These two enzymes are present in high concentrations in the liver. Any enzyme we use for medical diagnosis is based on its tissue of origin. If that enzyme is found in high concentrations in the blood, it means that the tissue of origin is now injured, pathologically affected, and possibly, dead. In the case of any damaged tissue, its contents are leaked out into the blood. Both of these enzymes(AST and ALT) are found in high concentrations in the liver, therefore, high concentration of these enzymes in the blood is an indication of liver problems.

The difference between these two enzymes, is that **ALT is found in high concentration in the liver, more or less, is exclusively found in the liver.** Its concentration in other tissues, is not significant enough to be used in medical diagnosis (not diagnostically relevant). **So, if you want to diagnose any liver problems, you would depend mostly on ALT**. AST is present at a higher concentration than ALT in the liver, but since it's found in other tissues, it's less specific. **AST will be detected first due to its high concentration. Therefore, ALT is more specific to the liver, AST is more sensitive to liver disease.**

Is transamination the only way to extract the amino group from amino acids? No, we can also extract the amino group via a process called deamination. We have different deaminases used to extract this amino group from amino acids, but not all of them work efficiently or benefit us.

*Oxidative deamination occurs mainly with one amino acid which we will discuss shortly

So, the second process we use to remove the amino group of amino acids is Oxidative deamination. The amino group is removed directly from the amino acid, without

attaching it to a second structure. This will produce free ammonia (NH3). This process is available for more than one amino acid. However**, the only amino acid which can undergo rapid oxidative deamination is glutamate**. So, the enzyme which removes the amino group is called glutamate dehydrogenase, because it is an oxidative deamination process.

Watch how the pieces of the puzzle fit together:

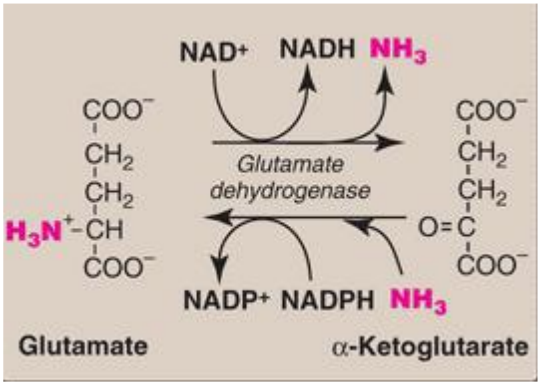
*Most transaminases are specific for a-ketoglutarate

*Most transaminases remove the amino group of amino acids and place it on a-ketoglutarate to produce glutamate.

*And glutamate is the only amino acid which can undergo rapid oxidative deamination

*So, we are funneling most amino acids to become glutamate, then we will deal with the glutamate on its own.

*We deaminate glutamate and remove the amino group, so it can become free ammonia resulting in a-ketoglutarate, its corresponding keto acid.

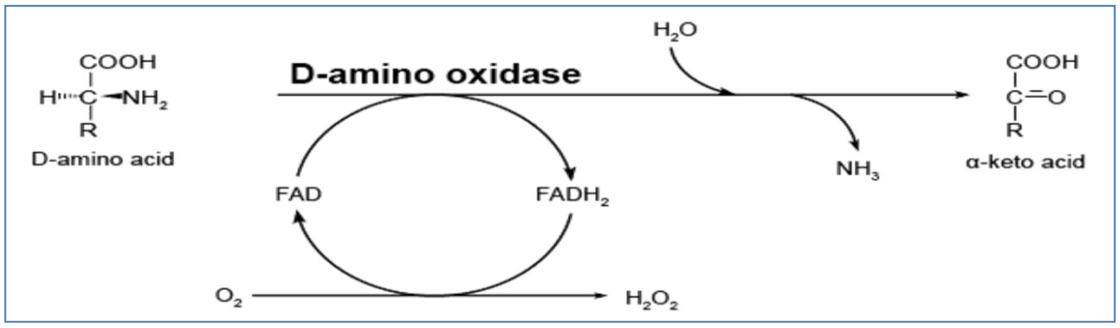


The enzyme used is glutamate dehydrogenase. This conversion process requires a co-enzyme called NAD+. NAD+ is required to convert glutamate to a-ketoglutarate. This reaction is reversible. The equilibrium constant is also almost 1. This means that if we have a large amount of a-ketoglutarate, we can reverse the reaction. The co-enzyme used in the reverse reaction is NADP+ and NADPH. Why are they different from one another? For the sake of the regulatory value. We have two enzymes used to control the reaction for better regulation .

The second enzyme used in removing amino group is D-amino acid oxidase. The interesting thing about this enzyme is the fact that it says D-amino acid, not L-amino acid. All the naturally occurring amino acids in our body are L-amino acids. So, why do we need D-amino acid oxidase? This is because plants and microorganisms have D-amino acids.

Essentially we are taking in food and microorganisms are everywhere, so to maximize the benefit of breaking down of proteins from the microorganisms, we should deal with the D-amino acids. D-amino acid oxidase converts the D-amino acid to its corresponding keto acid. Once again, the free amino group is released as ammonia. This is an oxidative

deamination reaction and results in the formation of hydrogen peroxide. The a-keto acid we ended up with, from D-amino acid, can be reaminated. Add another amino group to it and it becomes an L-amino acid. Once it's an L-amino acid, it can be used for protein synthesis. This enzyme is FAD-dependent peroxisomal enzyme.



So far, we've concluded that: Most amino acids will be funneled to donate their amino groups to α-ketoglutarate. α-ketoglutarate will be converted to glutamate. Then, glutamate dehydrogenase will extract the amino group from glutamate producing α-ketoglutarate and free ammonia.

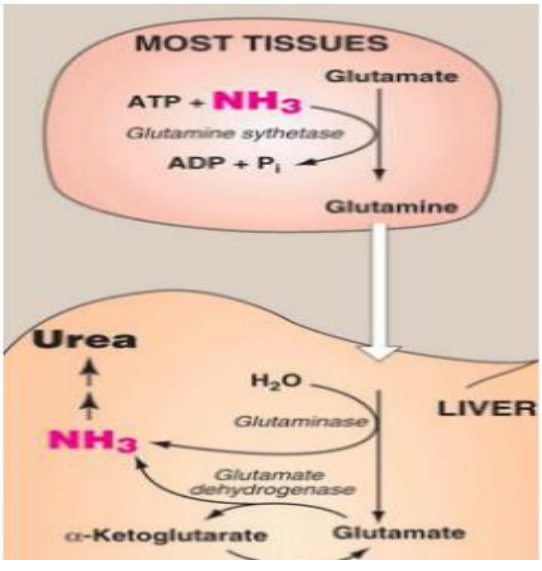
How do we deal with the free ammonia in tissues?

Ammonia is very toxic, especially to the CNS. A high concentration of ammonia in your blood is a medical emergency. The patient would need a hemodialysis. Since it is very toxic, it cannot be transferred through the blood. The fate of ammonia is conversion to urea. So we convert the toxic ammonia into the non-toxic compound, urea. Urea can be transferred through the blood and secreted through the urine.

In which tissue is urea made? The liver. The Urea Cycle cannot occur anywhere, but in the liver. All cells produce ammonia, but since the Urea Cycle can only occur in the liver, all ammonia must be transferred there.

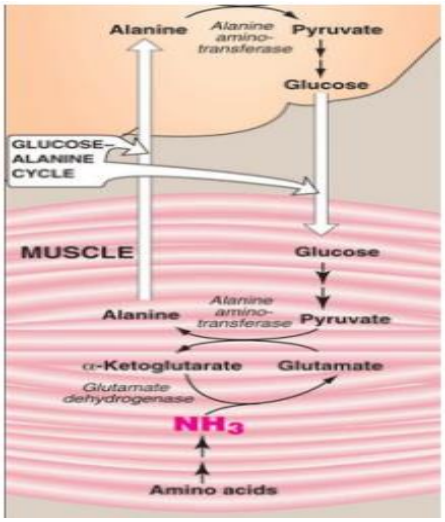
How do we transfer toxic ammonia from all cells to the liver, to be converted into Urea?

We have two methods:



1) **Attach this free ammonia to glutamate.** When glutamate is attached to an amine group (the free ammonia) , it is called glutamine. The enzyme that converts glutamate and ammonia into glutamine is called glutamine synthatase. This enzyme is available in all tissues. Glutamine is a safe alternative which can be transferred through the blood. Once

glutamine reaches the liver, it is broken down by glutaminase into glutamate and free ammonia.



2) **The Glucose-Alanine Cycle**: This occurs mainly between muscles and the liver. Glycogen, present in the liver, breaks down and gives you glucose. Glucose is transferred from the liver to muscles. The muscles utilize glucose by glycolysis, where the glucose is converted to pyruvate. By using ALT, pyruvate is converted to Alanine. ALT cannot take the amino group from free ammonia, so it takes it from glutamate, which is then converted to a-ketoglutarate. So, pyruvate is converted to alanine, alanine can be transferred safely via the blood to the liver. There, we have another transamination process, where alanine is converted to pyruvate and we end up with glutamate, which undergoes oxidative deamination to produce free ammonia.

So all amino acids in different tissues will have their amino groups taken out and given to a-ketogluturate, which will become glutamate. Glutamate dehydrogenase will come take out the free ammonia**. Free ammonia is hard to deal with in tissues, so we either attach it to glutamate to form glutamine or to pyruvate, to form alanine. Then they (glutamine and alanine) are transferred to the liver.** In the liver, we extract the free ammonia and convert it to urea, via the Urea cycle.

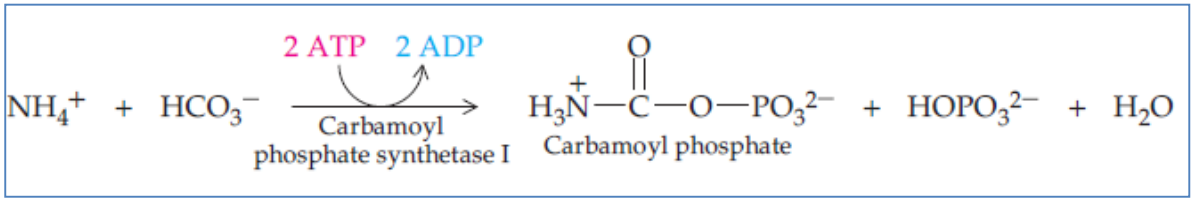
Humans have a Urea cycle, but fish don't, despite the fact that they also produce ammonia. Fish just release the ammonia into the water. The water is in such high amounts, in which the toxic ammonia is diluted and loses its toxicity. We would need high amounts of fluids to be able to live without the urea cycle, but our body wouldn't allow these large amounts. Our blood comes in relatively small amounts; not nearly enough to dilute ammonia.

Where does the Urea cycle occur? Only in the liver.

After urea is produced, it is transported to the kidnys, followed by the urinary bladder, then it leaves the body. The Urea Cycle accounts for 90% of the nitrogen containing components of the urine. In the urea cycle, you have the amino groups from the ammonia you've collected in the liver. You also have the CO_2 which we've gotten out of different metabolic processes.

A)Synthesis of the intermediate:

1) Combining CO_2 and free ammonia gives you Carbamoyl Phosphate (which has CO2, ammonia and phosphate group) and this reaction is done using the enzyme Carbamoyl phosphate synthetase 1(You do not need to know the action of 2). This is a ligation process which requires energy in the form of 2 ATP molecules. **This is the rate-limiting step of the Urea Cycle, the step where regulation occurs**. This occurs in the mitochondrial matrix.



2) **Carbamoyl phosphate will bind with a <u>basic</u> amino acid known as Ornithine (this amino acid is not from the 20 amino acids that are encoded in the gentic code), this reaction gives Citrulline** (also a basic amino acid). **The enzyme used is Ornithine trans-carbamoylase or Ornithine carbamoyltransferase**. This step occurs in the mitochondria, as well.
***the first and the second steps occur in the mitochondria.**

3) **Citrulline leaves the mitochondria and enters the cytosol**, where the remaining reactions will occur.

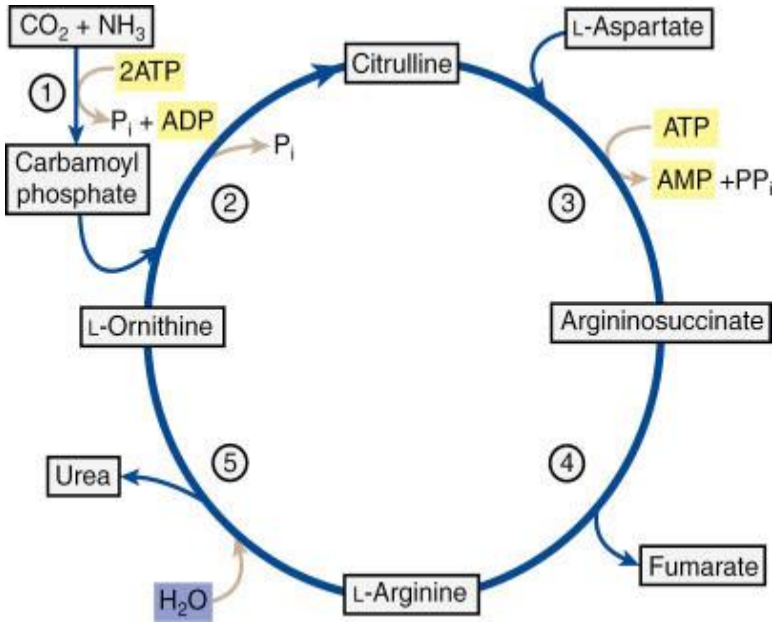
4) **In the cytosol, we have aspartate. Aspartate joins with Citrulline to make a compound called argininosuccinate** (a combination of the structure of arginine and succinate**). The enzyme used to catalyze this reaction is argininosuccinate synthetase**. **Argininosuccinate is the intermediate of the urea cycle.**

<u>B)Degradation of the intermediate</u>(to give back one of the starting materials→Ornithine)

5) The enzyme**, argininosuccinate lyase breaks down argininosuccinate into Arginine and** a double bond structure which was part of argininosuccinate, which is **fumarate.**

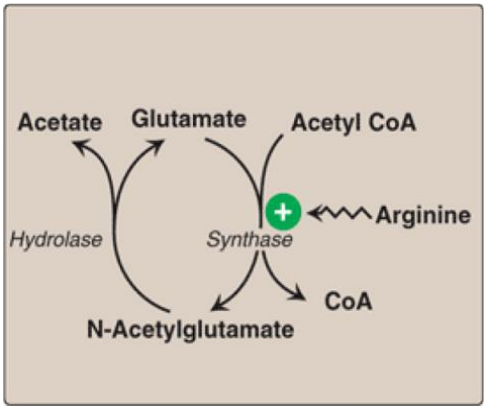
6) **Breaking down of Arginine into Urea and Ornithine by the enzyme Arginase.**

*Note: All the steps that came before the production of arginine could have occurred in any tissue, but the Urea Cycle occurs exclusively in the liver because the last step of **converting Arginine into Urea and Ornithine occurs via Arginase, which is ONLY present in the liver.** Without arginase, there is no urea cycle.



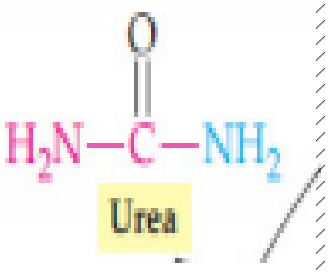
KEY TO ENZYMES (Circled Numbers)
1. Carbamoyl-phosphate synthase (ammonia)
2. Ornithine carbamoyltransferase
3. Argininosuccinate synthase
4. Argininosuccinate lyase
5. Arginase

Regulation of the Urea Cycle :



Occurs through using N-**acetylglutamate as a positive allosteric activator** for the cycle. It **will regulate the rate-limiting step which is carried by the enzyme CPS I (step #1).** More N-acetylglutamate stimulates the Urea Cycle, while less N-acetylglutamate slows it down. N-acetylglutamate increases after meals. When we've had a protein rich meal, by default, as an amino acid, arginine will increase. Arginine and glutamate will react, using the enzyme N-acetylglutamate synthase, and produce more N-acetylglutamate. (protein-rich diet, more arginine, more N-acetylglutamate, more urea cycle).

The only difference between the structure of aspartate and fumarate+succinate is that aspartate has an amine group. When aspartate combines with citrulline, aspartate sticks out of the carbon skeleton of citrulline, in argininosuccinate, and looks just like fumarate. The remaining amino group has to be in the final product; urea. Urea is basically a carbonyl group with two amino groups. →



The carbon group originally from carbon dioxide. One nitrogen group originally comes from the aspartate, which was oxalacetate, because it received an amino group from glutamate so it's the precursor of one of the amino groups in the urea. The second nitrogen comes from ammonia, itself. The ammonia comes from the oxidative deamination of glutamate. So, **glutamate is the precursor of both ammonia**.

*When we made carbamoyl phosphate, we used two ATP's.

*When aspartate entered the cycle, we used another ATP, which broke down to AMP, giving two phosphates (pyrophosphate) instead of one. These two phosphates will break down one again, to give us even more energy.

Net Result of the Urea Cycle:

*So how many ATPs did we utilize in this cycle? 3

*How many high-energy bonds did we break? 4 (2 for the first two ATPs and two from the third, which gave 2 phosphate groups)

*Production of fumarate (which comes from aspartate as a structure).

It's not important for urea to be in blood, just because it's transported safely. Some urea turns back into ammonia. Humans can not convert urea back into ammonia. As soon as ammonia is converted into urea, there is no turning back. However, some bacteria can convert urea to ammonia. This is not a problem in healthy adults. The urea will just go to the kidneys and leave the body. This is a problem when someone has renal failure. The urea concentration within their blood will be very high. They have a lot of urea in their circulation, so the amount of urea which can reach the intestines will also be very high. In the intestines, we have bacteria, and that bacteria will convert urea into ammonia, increasing the concentration of ammonia in the blood. Now, it's a serious problem. Ammonia is toxic. This is why people with renal failure take antibiotics; to decrease the amount of bacteria in their GI tract and limit their activity, so not as much ammonia is produced, which can harm the nervous system.

Hyperammonemia:

When does the concentration of ammonia in the blood increase?

When there is a high concentration of urea in the blood, giving bacterial urease a chance to act on it. Also, the liver is probably affected, for the concentration of urea in the blood, to increase, in the first place. The liver can either be affected by:

1) **Hereditary problems:** the gene for an enzyme could be deficient or faulty. **There are 5 enzymes in the urea cycle.** Each of these enzymes has a certain genetic deficiency. In every 1:30,000 births, you find someone with a deficiency in one of the urea cycle enzymes. 4 of the enzymes are affected by the autosomal recessive method of inheritance. 1 of them is X linked(carried on the X chromosome) and mostly affecting males because it will occupy their only copy of the X chromosome. **The X-linked deficiency is called Ornithine transcarbamoylase**, and it's the most common deficiency. **The normal concentration of serum ammonia is 5-50µmol/L**. **When there is a liver problem, the concentration may reach up to a 1000µmol/L.** This is a very high concentration and a medical emergency.

2) **Acquired problems**: such as hepatitis A,B, and C, alcoholism, cirrhosis, and fatty liver.

Dialysis is the immediate treatment for toxic materials in the blood. The long-term treatment is to decrease the amount of protein in your diet. Decreasing protein, decreases amino acids. Decreasing amino acids, decreases the amount of product from the urea cycle. Another long term treatment is to have many small meals, as not to increase the amino acid concentration all at once.