



Biochemistry

carbohydrates
proteins
isomers
ketone
starch
lipid
protein
amine

☒ Sheet

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Subject:	Biochemistry
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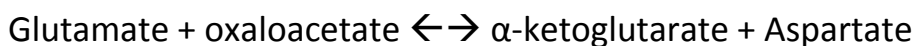
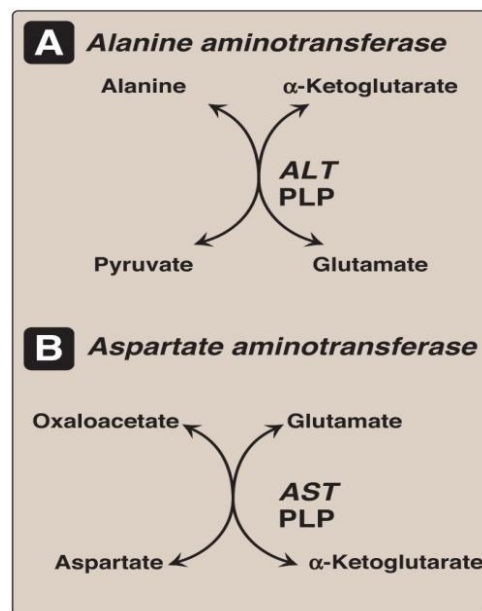
Last lecture:

We finished digestion and absorption of amino acids and then we said: in order for amino acid to be catabolized, the amino group should be removed. We said there are two mechanisms that work together in order to remove the amino group. The first one is *transamination* which happens for most amino acids and the second mechanism is *oxidative deamination*. Now, we will describe these two mechanisms in details:

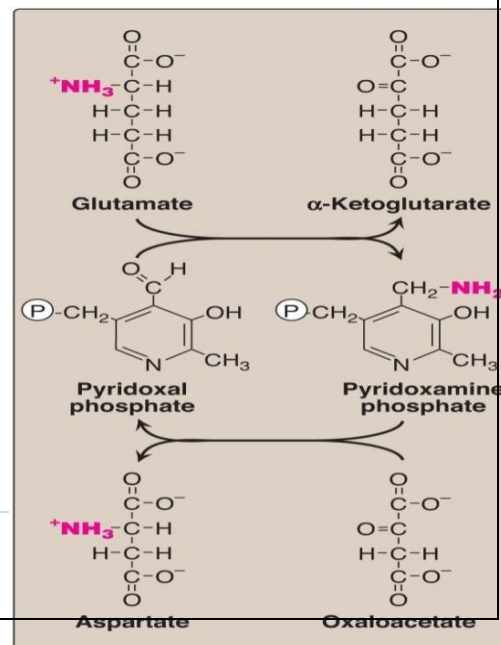
Transamination: the enzymes here are called transaminases and they require pyridoxal phosphate (PLP) as cofactor (which is the active form of vitamin B6). There are many of these transaminases, but we will talk about only two because of their diagnostic value. The first one is Alanine aminotransferase -or transaminase- (**ALT**). The second one is called Aspartate aminotransferase-or transaminase-(**AST**).

ALT: alanine can give amino group to α -ketoglutarate to form pyruvate and **glutamate** and the reaction can go in the reverse direction.

AST: the same thing happens here, but you should know that AST goes more in the direction of formation Aspartate because it is one of the sources of amino group in urea cycle (will be described later).



The mechanism of action for these enzymes: as we said, they require PLP as cofactor, but what is the role of PLP in the reaction? PLP is attached covalently to the side chain of lysine residue in the transaminase enzyme. The amino group is being transported to PLP (e.g. from alanine) then this amino group will be transferred from PLP to α -ketoglutarate for example. This will result in



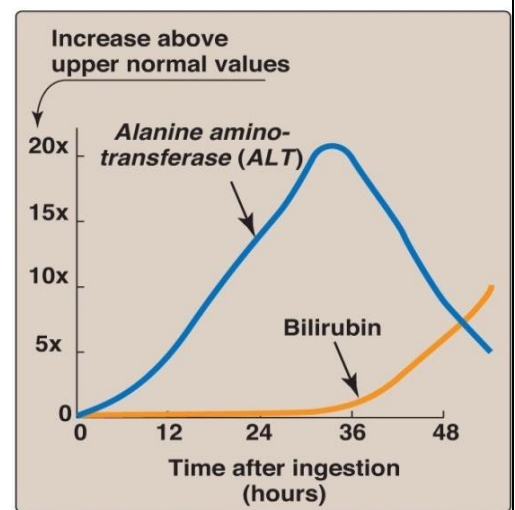
formation of amino acid glutamate and PLP is regenerated. (The figure shows AST action, which is similar).

Some information about ALT and AST:

-the level of them in serum is low because they are intracellular enzymes. They have a diagnostic value especially in liver diseases and even myocardial infarction. ALT and AST are both found in liver but AST is found in more amounts. So when there is damage in liver, the serum level of AST and ALT will be elevated. ALT is **more specific**, we use it more for liver disorders, but AST is also considered a measure in muscular destruction (for example after myocardial infarction), and also AST is **more sensitive** because as we said it's found in higher amounts.

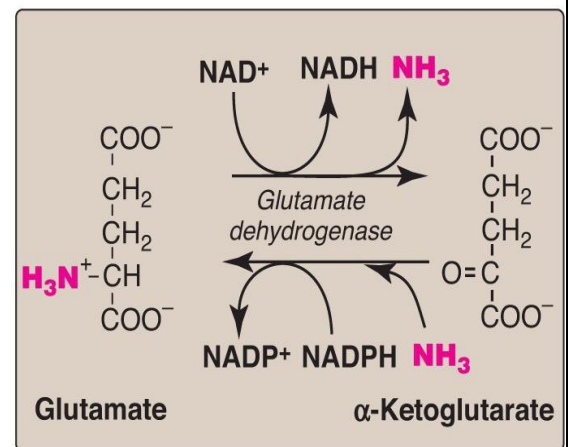
-Bilirubin is usually conjugated by the liver to be excreted, so if there is a problem in the liver, bilirubin concentration is elevated in the blood. Why do we mention bilirubin here? Because you should know that ALT and AST appear **earlier** than the increase in bilirubin. This is why they are always monitored.

We finished transamination mechanism which was the first mechanism.



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The second mechanism is *oxidative deamination*: in the process of transamination, Glutamate collects amino group from several amino acids. So there should be a mechanism where the amino group is removed from glutamate, therefore the enzyme *glutamate dehydrogenase* can oxidatively remove this amino group to form α -ketoglutarate. This enzyme uses NAD^+ or NADP^+ as cofactor (can use any of them) but in **oxidative** deamination NAD^+ is more used, and in the reverse reaction which is **reductive** amination NADPH is



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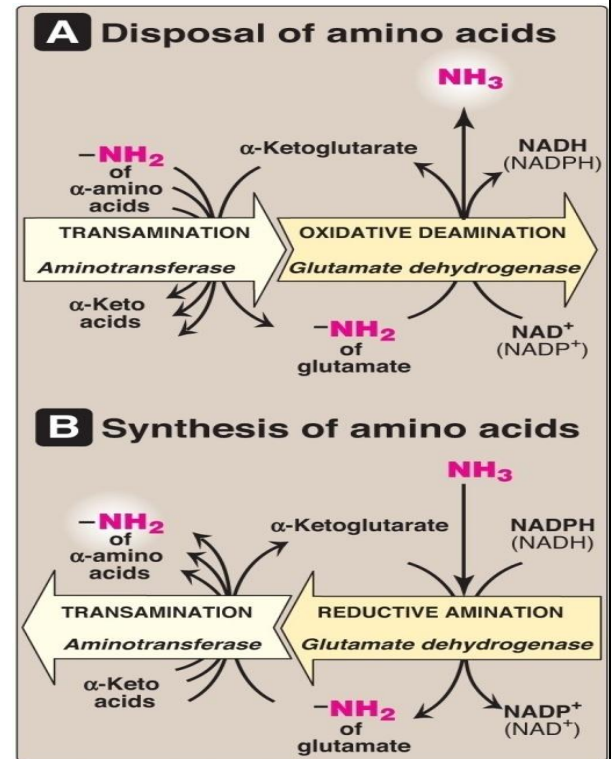
more used. The enzyme uses glutamate as a substrate and the products are **ammonia** and **α -ketoglutarate**. This enzyme is located in the mitochondria and oxidative deamination occurs primarily in liver and kidney.

Now look at the two mechanisms together: amino group of several amino acids can be disposed by linking to α -ketoglutarate and you get glutamate (transamination). This glutamate can undergo oxidative deamination and release ammonia. So this ammonia came from several amino acids via glutamate.

The same mechanism can be used to make **non essential amino acids**. All what we need is keto acid (carbon skeleton) which can be obtained from intermediates in pathways such as glycolysis,... this keto acid needs an amino group which is obtained from glutamate. We got glutamate when ammonia concentration is high from reductive amination of α -ketoglutarate. Ammonia + α -ketoglutarate will give us glutamate which can be used as donor of amino group to keto acids to form the needed amino acids.

The direction of reactions depends on the relative concentrations of glutamate, ammonia and α -ketoglutarate, and the ratio of oxidized to reduced coenzymes. For example, after rich meal of protein, glutamate level in the liver is elevated and direction proceeds in the direction of amino acid degradation and formation of ammonia. But if we want to drive reaction to glutamate synthesis, high ammonia level is required.

Allosteric regulators: Guanosine triphosphate is an allosteric **inhibitor** of glutamate dehydrogenase, whereas adenosine diphosphate (ADP) is an **activator**. Therefore, when energy levels are low in the cell, amino acid degradation by glutamate

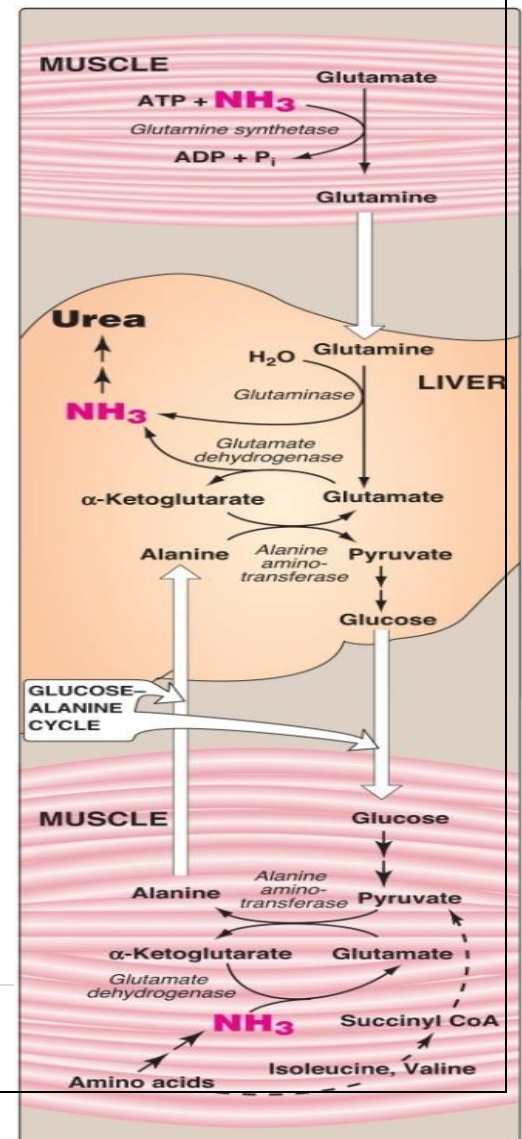


dehydrogenase is high, facilitating energy production from the carbon skeletons derived from amino acids.

*Amino acids that is found in proteins are L-amino acids, but we ingest some D-amino acids from plants, fungi ... these amino acids can be metabolized by enzyme called *D-amino acid oxidase* (DAO). This enzyme is FAD dependent enzyme that catalyzes the oxidative deamination of D-amino acids. DAO produces α -keto acid, ammonia and hydrogen peroxide. The resulted α -keto acid continues catabolism or may be reaminated and the result molecule is L-amino acid.

Transport of ammonia to the liver: the amino group is removed in the form of ammonia which is **toxic** molecule, so it must not be let free in our system. It is very toxic to brain. The level of ammonia in the blood must be kept very low. Ammonia is produced by various tissues; it is converted to urea in the liver which is a safe molecule. There are two mechanisms to transport ammonia to the liver: (*Note: both mechanisms are seen in –but not restricted to- skeletal muscles*).

1-by forming **glutamine** (in most tissues): glutamine is formed by the enzyme glutamine synthetase that requires energy. Ammonia is linked to glutamate requiring ATP and the result molecule is glutamine. This molecule is made in most tissues especially in muscles, liver and CNS. So glutamine is an important transporter for ammonia. If we measure the levels of amino acids in the blood, we will notice that glutamine has the highest concentration because it transports ammonia. After formation, glutamine in extrahepatic tissues will go to liver and kidneys, but mainly liver. Before, we said that ammonia is converted to urea in the liver only, so what is the role of glutamine in kidneys? The enzyme glutaminase (which is found in both liver and kidney) cleaves glutamine to glutamate and ammonia. Kidney use ammonia in the **acid-base balance**, kidney uses ammonia to remove protons from urine by forming ammonium ion NH_4^+ . So in the kidney ammonia can buffer protons and is then excreted as ammonium ion.



In the liver, glutaminase acts then ammonia forms. This ammonia is converted to urea.

2-by Alanine (muscles): alanine formed by transamination of pyruvate. First, ammonia with α -ketoglutarate results in glutamate then transamination with pyruvate which results in alanine.

Branched chain amino acids (isoleucine, valine) are metabolized in **skeletal muscles** more than liver. When they are metabolized, they give succinyl CoA which at the end becomes pyruvate.

In the liver, alanine gives the amino group to α -ketoglutarate forming pyruvate and glutamate which gives ammonia. The resulted pyruvate may undergo gluconeogenesis and form glucose. That is why we call it ***glucose-alanine cycle***.

Then ammonia in the liver enters the urea cycle.

Urea cycle:

Urea compound: has two amino groups and carbonyl group. One amino group comes directly from *free ammonia* and the other one come from glutamate but directly from *aspartate*. The C and O (carbonyl) in the compound come from *bicarbonate*.

Reactions of cycle:

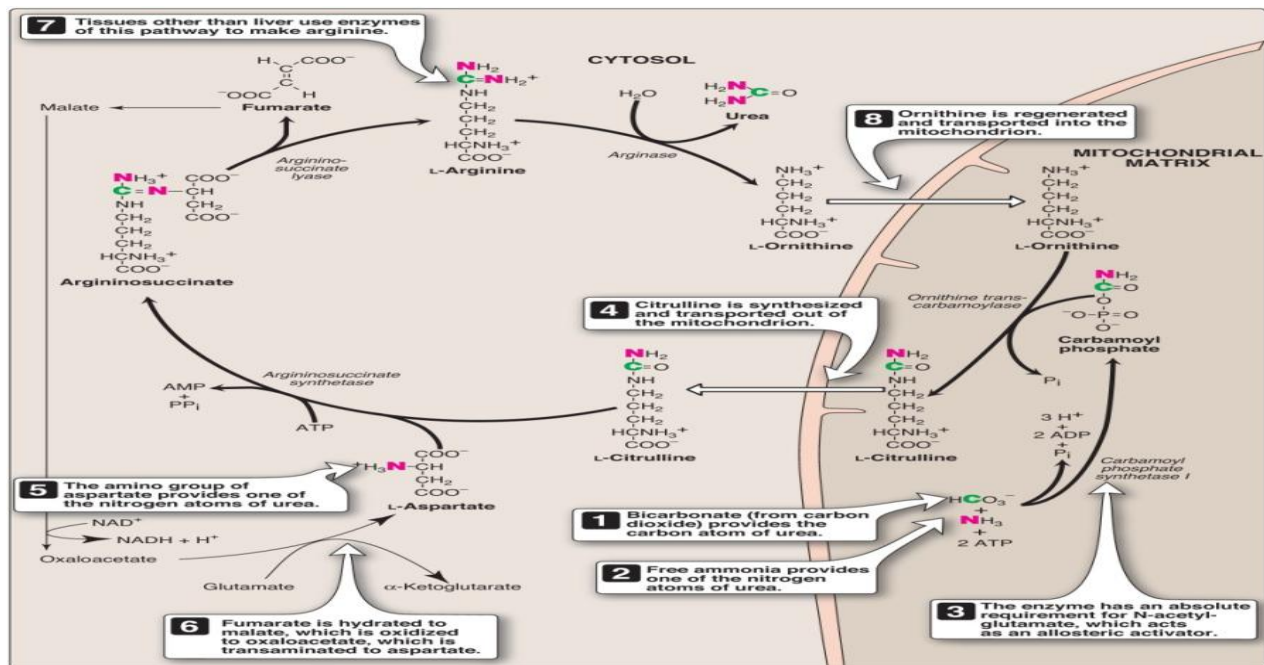
1-formation of carbamoyl phosphate: two-steps reaction. First, there is **activation** of CO_2 that comes from bicarbonate by the enzyme carbamoyl phosphate synthetase 1 (CPS1) in the mitochondria. Secondly, the resulting active carbonate **links** to ammonia. The formed compound is ***carbamoyl phosphate***. This reaction requires two ATP molecules (one for activation and one for linking). CPS1 is activated by N-acetylglutamate which is made from glutamate and acetyl CoA by the enzyme N-acetylglutamate synthetase. N-acetylglutamate is an allosteric activator for the first enzyme in the urea cycle. Also N-acetylglutamate synthetase is activated by arginine (allosteric activator).

2-formation of citrulline: carbamoyl phosphate condenses with ornithine in the mitochondria by the enzyme ornithine transcarbamoylase (OTC). The result is citrulline which leaves the mitochondria. Now citrulline carries two out of the three groups that are present in urea. The third group which is the second amino group is taken from aspartate.

3-synthesis of argininosuccinate: argininosuccinate synthetase combines citrulline with aspartate to form argininosuccinate. This reaction requires ATP, producing AMP and PPi, but PPi will be hydrolyzed making this reaction irreversible. So in urea cycle, body breaks 4 high energy bonds. (3ATP (steps 1+3) → 2ADP+2Pi + AMP+ 2Pi).

4-cleavage of argininosuccinate: by the enzyme argininosuccinate lyase. This results in fumarate and arginine. Tissues other than liver can do all these steps and form arginine. The resulted fumarate can be converted into malate then by malate dehydrogenase into oxaloacetate which takes amino group from glutamate to form aspartate which enters urea cycle again.

5-cleavage of arginine to ornithine and urea: by the enzyme arginase, this enzyme is found **only in liver**.



Urea cycle is induced by:

1-eating excess protein: because we metabolize proteins from diet.

2-in starvation: because we metabolize your own proteins to get energy.

Sources of ammonia:

1-major source is amino acids.

2-form bacterial action in intestine: these bacteria have the enzyme **urease**. The ammonia that results from urease is absorbed from the intestine and goes to liver

where it will be removed by forming urea. That is why **patients** with kidney problems, which cause more urea to the intestine, **more ammonia will be formed**. So we should give them antibiotics to kill the bacteria in intestine to stop the increasing in ammonia (because this ammonia will return back to the blood).

3-from amines: from diet and monoamines (hormones and neurotransmitters) by the action of *Mono Amine Oxidase (MAO)*.

4-from purines and pyrimidines

Hyperammonemia:

It is a dangerous case and requires special care. Normal level of ammonia in blood is (5-35 **micro** mol/L). when liver function is compromised, ammonia blood level can rise above 1000 micro mol/L. signs of hyperammonemia: tremors, slurring of speech, blurring of vision and very high concentrations of ammonia can cause coma and death.

Two types of hyperammonemia:

1-acquired hyperammonemia: liver diseases including: viral hepatitis, cirrhosis, alcoholism.

2-congenital hyperammonemia: all urea cycle enzymes deficiencies have been observed. The frequency of deficiency of the **all enzymes together** is 1:25000 live births. The most common deficiency is due to second enzyme "ornithine transcarbamoylase". This enzyme is X-linked, so most cases where seen in males. But female carriers may become symptomatic. Arginase deficiency is less severe because arginine already have trapped two ammonia, so symptoms in it is mild and excess arginine can be excreted in urine.

Treatment of hyperammonemia: 1-**Restriction** of protein diet.

2-some drugs can be used: for example phenylbutyrate, given orally, is converted to phenylacetate which condenses with glutamine to form phenylacetylglutamine which is excreted.

*****past papers*****

1- A patient who has a glutamine synthetase deficiency would have all of the following EXCEPT:

- A. Glutamate amination to glutamine is compromised
- B. Transport of ammonia from most tissues to liver is hindered
- C. Toxic levels of ammonia may accumulate in the patient's tissues and/or blood
- D. Transport of ammonia from muscle cells to the liver is not affected
- E. Transamination of α -ketoglutarate to glutamate is downregulated

2-which one of the following is wrong about urea cycle:

- a-it involves breaking of 4 high energy bonds
- b-formation of citrulline happens in the mitochondria
- c-it happens only in the liver
- d-starvation induce urea cycle.
- e-none of the above

3- 2 nitrogens of urea are from glutamate through:

- a-oxidative deamination
- b-transamination
- c-condensation
- d-a+b
- e-none of the above

4-which one of the following is true about first step in urea cycle:

- a-it involves breaking two high energy bonds and formation of AMP
- b-after it, we get molecule with two groups of amine.
- c-it involves breaking two high energy bonds and formation of two ADP
- D-after it, we get molecule with two carbonyl groups.
- e-none of the above.

5-which of the following are the products of ALT:

- a-aspartate and glutamate
- b-alanine and glutamate
- c-pyruvate and glutamine
- d-pyruvate and glutamate
- e-alanine and pyruvate

answers: 1/e,,,,,2/e,,,,,3/d,,,,,4/c,,,,,5/d

THE END