Transamination: the transmission of amino groups to glutamate

The first step in the catabolism of most amino acids is the transfer of their ⟨-amino group to ⟨-ketoglutarate. The products are an ⟨-keto acid derived from the amino acid and glutamate The acceptor of the amino group is almost always ⟨-ketoglutarate.. ⟨- Ketoglutarate plays a main role in amino acid metabolism by accepting the amino groups from most amino acids, becoming glutamate. Glutamate then can be oxidatively deaminated loosing amino group, or used as an amino group donor in the synthesis of nonessential amino acids. This transfer of amino groups from one carbon skeleton to another is catalyzed by a family of enzymes called aminotransferases, or transaminases. All amino acids, with the exception of lysine and threonine, participate in transamination. These two amino acids lose their ⟨-amino groups by deamination.

The two most important aminotransferase reactions are catalyzed by alanine aminotransferase (ALT) and aspartate aminotransferase (AST),

Alanine aminotransferase (ALT): ALT is present in many tissues. The enzyme catalyzes reversible transfer of the amino group of alanine to ⟨-ketoglutarate, resulting in the formation of pyruvate and glutamate. Glutamate, in effect, acts as a “collector” of nitrogen from alanine.

Aspartate aminotransferase (AST): AST reversible transfers amino groups from glutamate to oxaloacetate, forming aspartate, which is used as a source of nitrogen in the urea cycle.

All aminotransferases require the coenzyme pyridoxal phosphate, a derivative of vitamin B_6, which is covalently linked to the ∑-amino group of a specific lysine residue at the active site of the enzyme. Transamination reactions function in both directions. After consumption of a protein-rich meal amino acid degradation begins with removal of ⟨-amino group. But when the supply of amino acids from the diet is not adequate to meet the synthetic needs of cells, amino acids are synthesized through addition of amino groups to the carbon skeletons of ⟨- keto acids.

Diagnostic value of plasma aminotransferases: Aminotransferases are normally intracellular enzymes. Their activity in the norm is low in the plasma. The presence of elevated plasma levels of aminotransferases indicates damage to cells rich in these enzymes. Two aminotransferases—AST and ALT— have the diagnostic value when they are found in the plasma. Plasma AST and ALT are elevated in nearly all liver diseases. ALT is more specific than AST for liver disease, but the latter is more sensitive because activity of AST the liver is higher.

Glutamate dehydrogenase participates in the oxidative deamination of amino acids

In contrast to transamination reactions that transfer amino groups, oxidative deamination by glutamate dehydrogenase results in the liberation of the amino group as free ammonia (NH_3). These reactions occur primarily in the liver and kidney. They provide 〈-keto acids, that can enter the central pathway of energy metabolism, and ammonia, which is a source of nitrogen in urea synthesis. As described above glutamate is unique in that it is the only amino acid that undergoes rapid oxidative deamination—a reaction catalyzed by glutamate dehydrogenase. **Glutamate dehydrogenase is unusual in that it can use either NAD^+ or $NADP^+$ as a coenzyme. NAD^+ is then used primarily in oxidative deamination, and NADPH is used in reductive amination.** Guanosine triphosphate (GTP) is an allosteric inhibitor of glutamate dehydrogenase, whereas adenosine diphosphate (ADP) is an activator. Thus, 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.

D-Amino acid oxidase: D-Amino acids are found in plants and in the cell walls of microorganisms, but are not found among mammalian proteins. D-Amino acids are

efficiently metabolized by the kidney and liver. D-Amino acid oxidase is an FAD-dependent peroxisomal enzyme that catalyzes the oxidative deamination of these amino acid isomers, producing ⟨-keto acids, ammonia, and hydrogen peroxide. The ⟨-keto acids can enter the general pathways of amino acid metabolism, and be reaminated to L-isomers, or catabolized for energy.

L**-Amino acid oxidase:** is an FMN-dependent peroxisomal enzyme that catalyzes the oxidative deamination of these amino acid isomers, producing ⟨-keto acids, ammonia, and hydrogen peroxide.

In most tissues for the transport of ammonia from the peripheral tissues to the liver (for its ultimate conversion to urea) *glutamine synthetase* is used. This enzyme combines ammonia (NH_3) with glutamate to form glutamine—a nontoxic transport form of ammonia. This glutamine is transported in the blood to the liver where it is cleaved by *glutaminase* to produce again glutamate and free ammonia. Muscle uses the second transport mechanism with transamination of pyruvate (the end product of aerobic glycolysis) to form alanine. Alanine then is transported by the blood to the liver, where it is again converted to pyruvate.

Urea cycle

Urea is produced by the liver, it is the major disposal form of amino groups derived from amino acids. One nitrogen of the urea molecule is supplied by free ammonia,

and the other nitrogen - by aspartate. The carbon of urea is derived from CO_2. Ammonia sourses primarily are: amino acids and their amids, biogenic amines, purines, pyrimidines.

The first two reactions of urea cycle occur in the mitochondria, whereas the remaining cycle enzymes are located in the cytosol.

1. Formation of carbamoyl phosphate: Formation of carbamoyl phosphate by *carbamoyl phosphate synthetase I* is driven by cleavage of two molecules of ATP. Carbamoyl phosphate synthetase I requires **N-acetylglutamate** as a positive allosteric activator. (Reminder: Carbamoyl phosphate synthetase II participates in the biosynthesis of pyrimidines, uses glutamine as the nitrogen source, and occurs in the cytosol).

2. Formation of citrulline: *Ornithine transcarbamoylase* (OTC) transferres the carbamoyl portion of carbamoyl phosphate to ornithine. The high-energy phosphate is released as P_i. The reaction product, **citrulline**, is transported to the cytosol.

3. Synthesis of argininosuccinate: *Argininosuccinate synthetase* combines citrulline with aspartate to form **argininosuccinate**. The ⟨-amino group of aspartate provides the second nitrogen that is incorporated into urea. This reaction is driven by the cleavage of ATP to adenosine monophosphate (AMP) and pyrophosphate. This is the third molecule of ATP consumed in the formation of urea.

4. Cleavage of argininosuccinate: Argininosuccinate lyase cleaves argininosuccinate with yielding **arginine** and fumarate. The arginine formed by this reaction is a precursor of urea. Fumarate produced in the urea cycle is hydrated to malate, providing a link with several metabolic pathways. For example, the malate can be transported into the mitochondria via the malate shuttle, reenter the tricarboxylic acid cycle, and get oxidized to oxaloacetate (OAA), which can be used for gluconeogenesis. Alternatively, the OAA can be converted to aspartate via

transamination, and can enter the urea cycle

5. Cleavage of arginine to ornithine and urea: *Arginase* cleaves arginine to ornithine and **urea**. Arginase occurs almost exclusively in the liver. Thus, only the liver can synthesize urea. Urea diffuses from the liver, and is transported to the kidneys, where it is filtered and excreted in the urine. A portion of the urea diffuses from the blood into the intestine, and is cleaved to CO_2 and NH_3 by bacterial **urease**. In patients with kidney failure, plasma urea levels are elevated, promoting a greater transfer of urea from blood into the gut. The intestinal action of urease on this urea becomes a clinically important source of ammonia, contributing to the *hyperammonemia* often seen in these patients. Oral administration of neomycin reduces the number of intestinal bacteria responsible for this NH_3 production.

Overall stoichiometry of the urea cycle:

$$\text{Aspartate} + NH_3 + CO_2 + 3\ \text{ATP} + H_2O \longrightarrow \text{urea} + \text{fumarate} + 2\ \text{ADP} + \text{AMP} + 2\ P_i + PP_i$$

Four high-energy phosphate bonds are consumed in the synthesis of each molecule of urea; therefore, the synthesis of urea is irreversible.

The intrahepatic concentration of N-acetylglutamate increases after ingestion of a protein-rich meal. This leads to an increased rate of urea synthesis.

Hyperammonemia has a direct neurotoxic effect on the CNS, which include tremors, slurring of speech, somnolence, vomiting, cerebral edema, and blurring of vision. At high concentrations, ammonia can cause coma and death. The two major types of hyperammonemia are: acquired and congenital**.**

Liver disease is a common cause of hyperammonemia in adults, and may be due to alcohol. The conversion of ammonia to urea is impaired, leading to elevated levels of ammonia. Genetic deficiencies of each of the five urea cycle enzymes leads to hyperammoniemia during the first weeks following birth. Ornithine *transcarbamoylase* deficiency, which is X-linked, is the most common of these

disorders, predominantly affecting males, although female carriers may become symptomatic. The hyperammonemia seen with *arginase* deficiency is less severe because arginine contains two waste nitrogens and can be excreted in the urine. Historically, urea cycle defects had high morbidity (neurological manifestations) and mortality. Treatment included restriction of dietary protein in the presence of sufficient calories to prevent catabolism. Administration of compounds that bind to amino acids, producing molecules that are excreted in the urine, has improved survival. For example, phenylbutyrate given orally is converted to phenylacetate. This condenses in organism with glutamine to form phenylacetylglutamine, which is excreted.