

Welcome for the module from the subjects Zoology from the program, Bachelors of Science for 3rd year. Myself, Dr. K. K. Therisa, assistant Professor, Dhempe College of Arts and Science Miramar, Goa.

The paper is Biochemistry and Metabolic processes from ZOC 106 and the name of the unit is Amino acid metabolism and the module name is urea cycle.

The outline of this module. First, the urea cycle in ureotelic animals and then we will discuss in detail the process of urea cycle.

The learning outcome is the student will be able to understand the importance of urea cycle in ureotelic animals and learn the process of urea cycle.

The nitrogen from the amino group of a protein is excreted in three forms in different living organisms. For example, in ammonotelic animals- It is excreted in the form of ammonia, and it is seen in some of the aquatic vertebrates like fishes. The other group of animals are known as ureotelic animals as they excrete urea as excretory product, and examples are many of the terrestrial vertebrates. Whereas a third group is the Uricotelic animals wherein they excrete uric acid and the examples are reptiles and birds.

The urea cycle is important as far as ureotelic animals are concerned. The ammonia in the mitochondria of a liver cells has been converted to urea via urea cycle. This pathway was discovered by Hans Adolf Krebs and Kurt Henseleit in 1932. In fact, urea cycle is the first cyclic metabolic pathway to be discovered. There are three important amino acids which plays a very important role in accelerating the rate of the cycle. Those are ornithine, citrulline and arginine. A mole of ornithine combines with one mol of ammonia and one of the carbon dioxide molecules, to form a Citrulline molecule.

A second amino group is added to citrulline form arginine. And arginine is hydrolyzed to yield urea with concomitant regeneration of ornithine by enzyme arginase.

The ornithine is now ready for next turn of urea cycle. Urea, passed via the bloodstream to kidney and then excreted into urine. Urea cycle spans 2 cellular compartments, very important because it requires a mitochondrial matrix and the cytoplasm to complete the process. As some of the important enzymes are found restricted only to the mitochondrial matrix, whereas some of the enzymes are in the cytoplasm. It begins its process in mitochondria and then three steps occurs inside the cytosol. The first amino group in the form of ammonia, enter urea cycle inside a mitochondria. Synthesis of 1 mole of urea requires 3 mol of ATP, One mole of ammonium and Aspartate along with five enzymes. Ammonia together with HCO₃ or carbon dioxide produced by the mitochondrial respiration, along with ATP, forms, are important intermediate called carbamoyl phosphate. By the action of mitochondrial enzyme known as carbamoyl phosphate synthetase 1. Carbamoyl phosphate synthetase one is active only in the presence of N-acetyl glutamate, as an allosteric activator for this enzyme and enhances the affinity of synthetase to the ATP molecule. Carbamoyl phosphate synthetase one is actually initiates the urea biosynthesis.

This is a chart which depicts, in brief, the urea cycle. You can see here is the cytosol and this is the mitochondria. One of the important processes is the formation of citrulline from the carbamoyl phosphate occurs inside the mitochondria as the enzyme carbamoyl phosphate synthetase, one is present inside the mitochondria. Later, the ornithine which enters inside the mitochondria combines with carbamoyl phosphate to form the first product, that is the citrulline. As citrulline moves out of the mitochondria forms a argininosuccinate when it combines with aspartate inside the cytosol. The Argininosuccinate further is transformed into arginine, wherein a fumarate molecule is been released in this process, which enters into the citric acid cycle. The arginine will again get transformed into a ornithine molecule along with it, it will release a urea which has two amino group. And this is how a urea been formed.

So the carbamoyl phosphate, which enters the urea cycle comprised of the following for enzymatic step: 1. is the formation of citrulline, 2. formation of argininosuccinate, 3. cleavage of argininosuccinate into the arginine and fumarate and 4. cleavage of arginine to ornithine and urea.

Altogether, we can say that five important steps are involved in urea cycle. Number one being the formation of carbamoyl phosphate and later the carbamoyl phosphate which enters into the cytoplasm. Further, four steps that takes place, and these are the ones.

Let us see one by one synthesis of citrulline. The carbamoyl phosphate donates its carbamoyl group to the ornithine molecule which has entered into the mitochondria from the cytoplasm to form a citrulline and This is the process where a phosphate, the inorganic phosphate, has been released and it has been catalyzed by an important enzyme called L-Ornithine transcarbamoylase. Citrulline released from the mitochondrial Matrix to the cytosol. And while the reaction occurs in the mitochondrial matrix, both formation of ornithine and subsequent metabolism of citrulline takes place in the cytosol. The entry of ornithine into the mitochondria and the exodus of the citrulline from the mitochondria involves a very important enzyme called permeases inside the mitochondrial inner membrane.

The Step 2 that is synthesis of argininosuccinate, which takes place now inside the cytosol, where in the Aspartate from the mitochondria transported to the cytosol, will condense with the ureido or also known as carbonyl group of citrulline to form a molecule known as argininosuccinate. Enzyme involved is argininosuccinate synthetase. And these are found inside this cytosol. It requires ATP, that cleaves into AMP and a pyrophosphate, and proceeds through a intermediate molecule which is known as citrullyl-AMP. In the beginning, subsequent displacement of AMP by aspartate then forms Arginino succinate synthetase links the aspartate and citrulline via amino group of aspartate and provides the second nitrogen of urea.

Step 3. Cleavage of argininosuccinate to arginine and fumarate:

Cleavage of argininosuccinate to arginine and fumarate is the important step as a fumarate at this point has been released, which can enter into the citric acid cycle. And this has been catalyzed by an enzyme known as argininosuccinate, at least as it is going to break these two molecules. Reversibly cleaves the argininosuccinate into the arginine and fumarate. The reaction proceeds with retention of all three nitrogens

in the arginine. And the release of the aspartate skeleton alone in the form of a fumarate which doesn't have an amino group. And this can enter easily into the citric acid cycle.

Whereas the final step that is the cleavage of arginine, ornithine and urea. Arginine so produced is cleaved by the cytosolic enzyme known as arginase into a urea and ornithine. So therefore, ornithine is regenerated back into the cytosol, which will be transported back into the liver mitochondria and initiate a second round of urea cycle so that continuously the nitrogen in the form of ammonia is being transformed into a urea so that a cell can avoid a toxic environment.

Ornithine and lysine are potent inhibitors of arginase and compete with arginine.

So this is the urea cycle.

Where in a mitochondria and the cytoplasm or cytosol.

Inside the mitochondria, the carbon dioxide which has entered along with the ammonia it combines and forms the carbamyl. Carbamoyl phosphate, with the help of an enzyme carbamoyl phosphate synthetase. So this combines with the ornithine which has entered inside the mitochondria from the cytosol and forms a molecule called citrulline that traverses out into the cytosol. Further, the citrulline will combine with the aspartate with the help of argininosuccinate synthetase enzyme get transformed into argininosuccinate molecule, and this molecule is cleaved by the Argininosuccinate lyase enzyme into arginine and a fumarate molecule. The fumarate molecule will enter into the citric acid cycle whereas the arginine which has the ammonia attached to it in the form of an amino group will get transformed into a urea molecule and again an ornithine. And the ornithine will be released. Into the cytosol, which will enter into the mitochondria to combine with carbamoyl phosphate molecule and to start with a second round of urea cycle.

These are the references which I use to prepare this module.

Thank you.