BCH 447 Practical Metabolism

Estimation Of Arginase Activity In Liver Extract

Objectives

Estimation of Arginase activity in liver extract

Introduction

- **Ammonia** is a product of oxidative deamination of amino acids.
- It is toxic in even small amount and it must be removed from the body.
- **Arginase** is one of the important enzymes in urea cycle which is the major disposal form of amino groups derived from amino acids and accounts for about 90% of the nitrogen-containing compounds of urine.
- **Urea cycle** catalyzed by a set of enzymes (**Five enzymes**) present in the liver, and then is transported in the blood to the kidneys for excretion.

Arginase

- The arginase enzyme catalyzes the final reaction in the **urea cycle**, the enzyme is present exclusively in the liver.
- Arginase catalyzes the hydrolytic cleavage of the guanidino group of **arginine** to regenerate **ornithine** and **urea**.

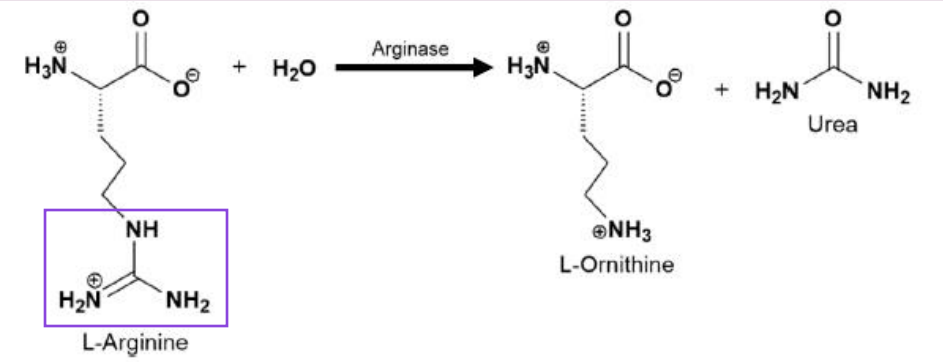

Figure 1. Chemical reaction of arginase

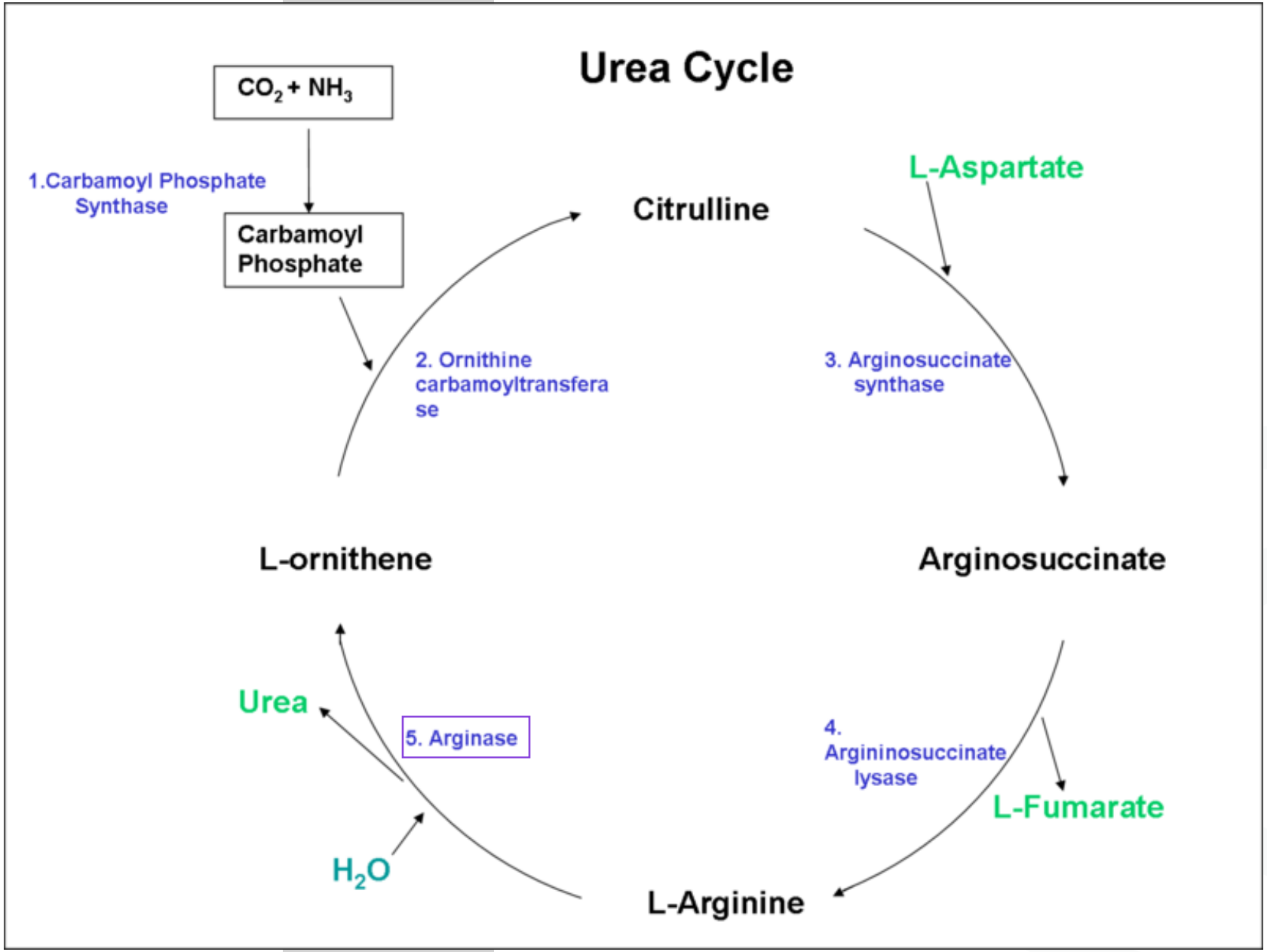

Figure 2. Urea cycle

Arginase

- Two isozymes of this enzyme exists:
  1. Arginase I **(In cytoplasm)** functions in urea cycle.
  2. Arginase II **(In mitochondria)** to regulate the arginine/ornithine concentration in the cell.

- **Arginase** requires a two-molecules metal of Co^{2+} and Mn^{2+} for it's activation while **ornithine** and **lysine** are potent inhibitors.

Preparation of crude arginase extract:

1. Weigh one fresh liver
2. Homogenate in a volume of cold potassium phosphate buffer (0.05 M, pH 7.4) equal to 3 times its wet weight.
3. The homogenate is centrifuged in the cold for 1 minute at 8000 rpm.
4. The supernatant contains the enzyme and must be kept cold.
5. Measure the extract volume .
6. Dilute the liver extract 1:5 with ice-cold buffer and use this diluted extract.

Principle (*of the used kit*):

The reagent used contains: **Urease, Glutamate Dehydrogenase, NADH, 2-oxoglutarate, buffers and stabilizers .**

1. **Reaction one:** Urea is hydrolysed in the presence of urease enzyme and water to yield ammonia and carbon dioxide.

$$\underset{\text{(Urea)}}{NH_2 - CO - NH_2} + H_2O \xrightarrow{Urease} \mathbf{2NH_3} + CO_2$$

2. **Second reaction:** The ammonia reacts with 2-oxoglutarate and reduced nicotinamide adenine dinucleotide (NADH) in the presence of glutamate dehydrogenase (GLDH) to yield glutamate and nicotinamide adenine dinucleotide (NAD).

$$\mathbf{2NH_3} + \text{2-oxoglutarate} + \mathbf{NADH + H^+} \xrightarrow{GLDH} \text{Glutamate} + \mathbf{NAD^+} + H_2O$$

The amount of the **urea** in the sample is proportionally related to the reduced absorbance at 340 nm as a result of **NADH oxidation** to NAD.

Method:

	Standard	Serum
Working reagent	1ml	1ml
Pre-warm at 37°C for 3 min. and add:		
Standard	0.01/10µl	-
Serum	-	0.01/10µl

1. After exactly **30 seconds,** read and record absorbance $\mathbf{A_1}$ against distilled water at **340 nm.**
2. At exactly **60 seconds** after A_1, read and record the absorbance $\mathbf{A_2}$ and determine $\Delta\mathbf{A}$ ($\mathbf{A_1}$-$\mathbf{A_2}$).

Calculations of the Results:

$$\text{-Urea (mmol/L)} = \frac{\Delta A\ (\text{Sample})}{\Delta A\ (\text{Standard})} \times \text{Std. Conc. (30)}$$

Calculations

Calculate the total arginase activity in the liver as micromoles of urea produced per gram of liver.

1. Urea produced in 1 minute = ------- mmol /min /0.01ml of liver extract.
2. Urea in 1ml of diluted liver extract = ------- x 100 = -------mmol/min/ml of diluted liver extract.
3. Urea concentration in 1ml of undiluted liver extract = ----- x 3 x 5
4. Urea concentration in micromoles = -------- x1000 = ------- micromoles/min/ml.
5. Total activity present in liver = --------- x total volume of liver extract (80ml) = ------- micromole.
6. **Arginase activity as (µmol/g) of liver** = total activity in liver/ wt of liver (20g).