Protein Turnover and Amino Acid Catabolism

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Protein Degradation

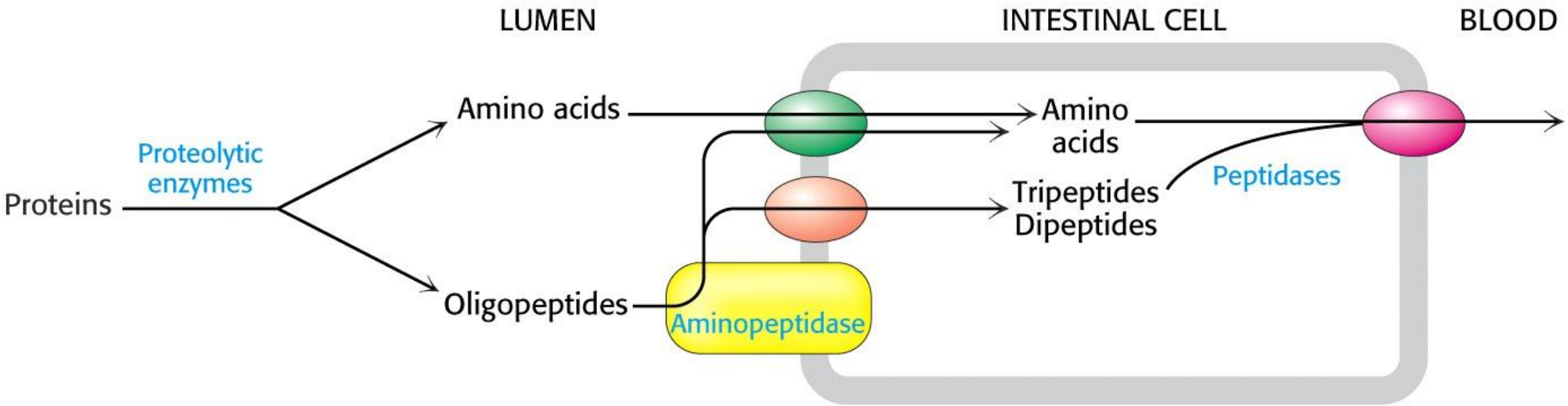
1.Dietary Protein Digestion

2.Cellular Protein Turnover

Dietary Protein Turnover

- Proteins digested to amino acids and small peptides in the stomach
- Acid environment denatures proteins making them more accessible to proteases
- Pepsin is a major stomach protease, has pH optimum of 2.0
- Protein degradation continues in the lumen of the intestine by pancreatic proteases
- Amino acids are then released to the blood stream for absorption by other tissues

Dietary Protein Turnover



Cellular Protein Turnover

- Damaged proteins need to be degraded
- Proteins involved in signaling are rapidly degraded to maintain tight regulation
- Enzymes are often degraded as part of a pathway regulatory mechanism (HGM-CoA Reductase)

Protein Turnover Rates Vary

- Proteins are constantly being degraded and resynthesized
- Ornithine decarboxylase has short half life 11 minutes (polyamine synthesis-impt in cell growth and diff)
- Hemoglobin and crystalline are very long lived protein
- N-terminal amino acid residue determines protein stability

TABLE 23.1 **Dependence of the half-lives of cytosolic yeast proteins on the nature of their amino-terminal residues**

Highly stabilizing residues ($t_{1/2}$ > 20 hours)			
Ala	Cys	Gly	Met
Pro	Ser	Thr	Val
Intrinsically destabilizing residues ($t_{1/2}$ = 2 to 30 minutes)			
Arg	His	Ile	Leu
Lys	Phe	Trp	Tyr
Destabilizing residues after chemical modification ($t_{1/2}$ = 3 to 30 minutes)			
Asn	Asp	Gln	Glu

Source: J. W. Tobias, T. E. Schrader, G. Rocap, and A. Varshavsky. *Science* 254(1991):1374.

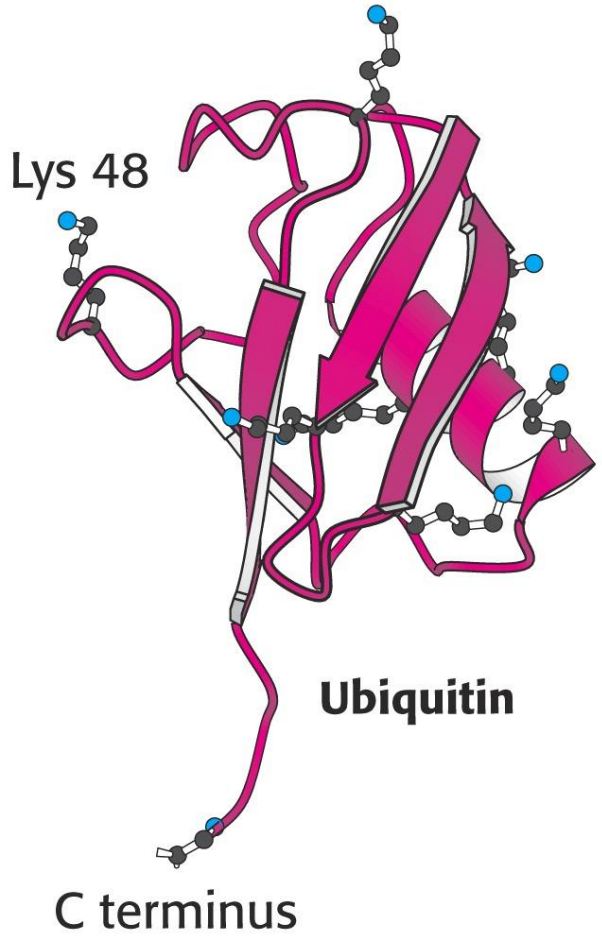
- Proteins are degraded into amino acids
- Amino acids used for synthesizing proteins are obtained by degrading other proteins
    - Proteins destined for degradation are labeled with *ubiquitin*
    - Polyubiquinated proteins are degraded by *proteasomes*
- Amino acids are also a source of nitrogen for other biomolecules

Lysosomal Hydrolysis

- Proteins to be destroyed are encapsulated in vesicles
- Proteins are deposited in lysosomes by the fusion of vesicles with the lysosomal membrane
- Lysosomal proteases degrade protein

Ubiquitin

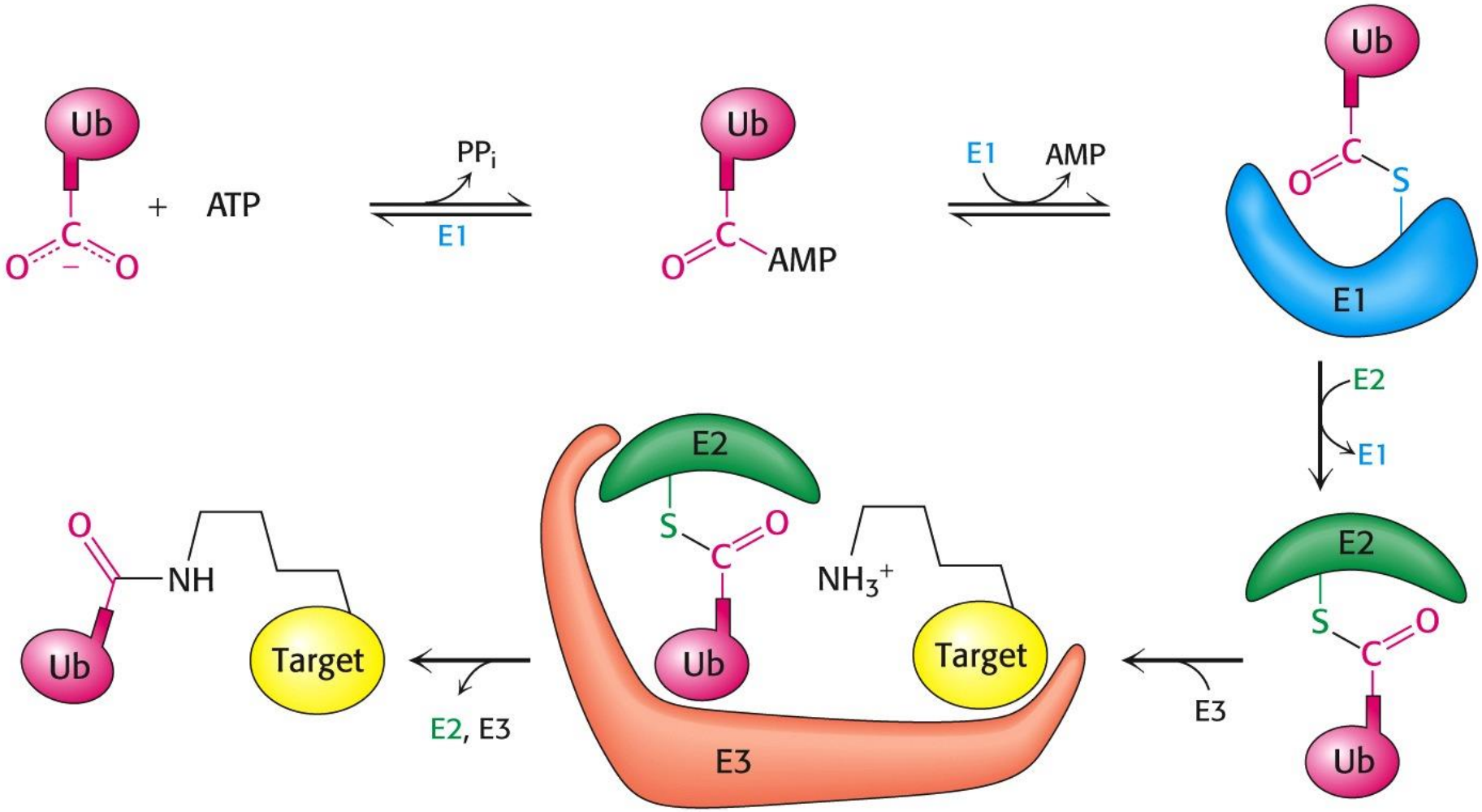
- Ubiquitin is a small protein (8.5 kD = 76 amino acids)
- Highly conserved among all Eukaryotes
- When covalently attached to a protein, ubiquitin marks that protein for destruction



Tagging of Protein

- The carboxyl-terminal glycine of ubiquitin covalently attaches to e-amino group of lysine residues on target protein
- Requires ATP hydrolysis
- Three enzymes involved:
  1) E1, ubiqutiin activating protein
  2) E2, Ubiquitin conjugating enzyme
  3) E3, ubiquitin-protein ligase.

Ubiquitin



Multiple Ubiquitins can be polymerized to each other.

What determines whether a protein will become ubiquinated?

- E3 enzyme are readers of N-terminal amino acid residues
- N-terminal amino acids determine stability of protein
- Also proteins rich in proline, glutamic acid, serine and threonine (PEST sequences) often have short ½ lives.
- Other specific sequences (e.g. cyclin destruction box) target proteins for ubiquitination

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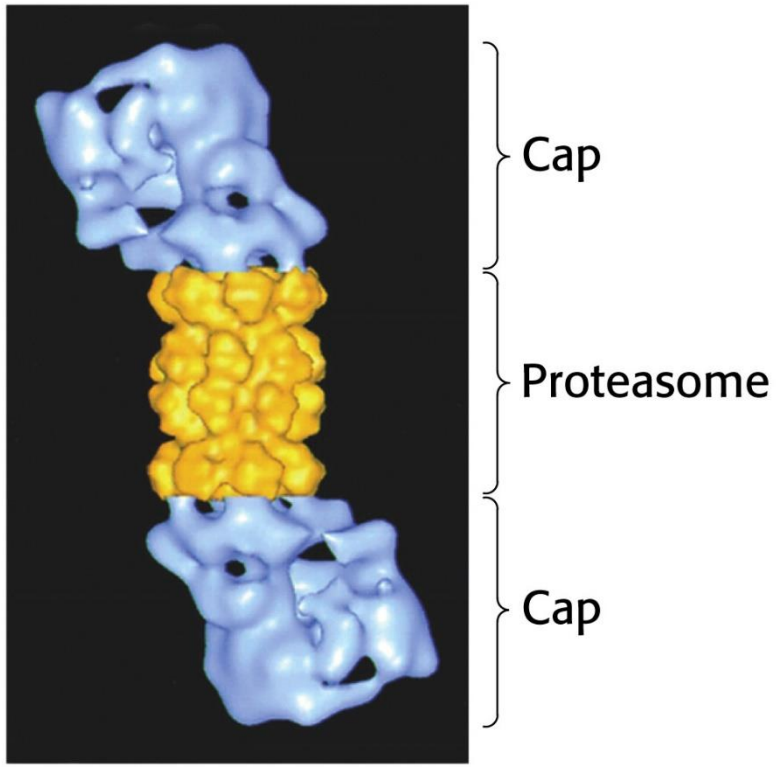
Pathological Condition Related to Ubiquination

- Human papilloma virus encodes a protein that activates a specific form of the E3 enzyme that ubiquitinates several proteins involved in DNA repair.
- Activation of this E3 enzyme is observed in 90% of cervical carcinomas.

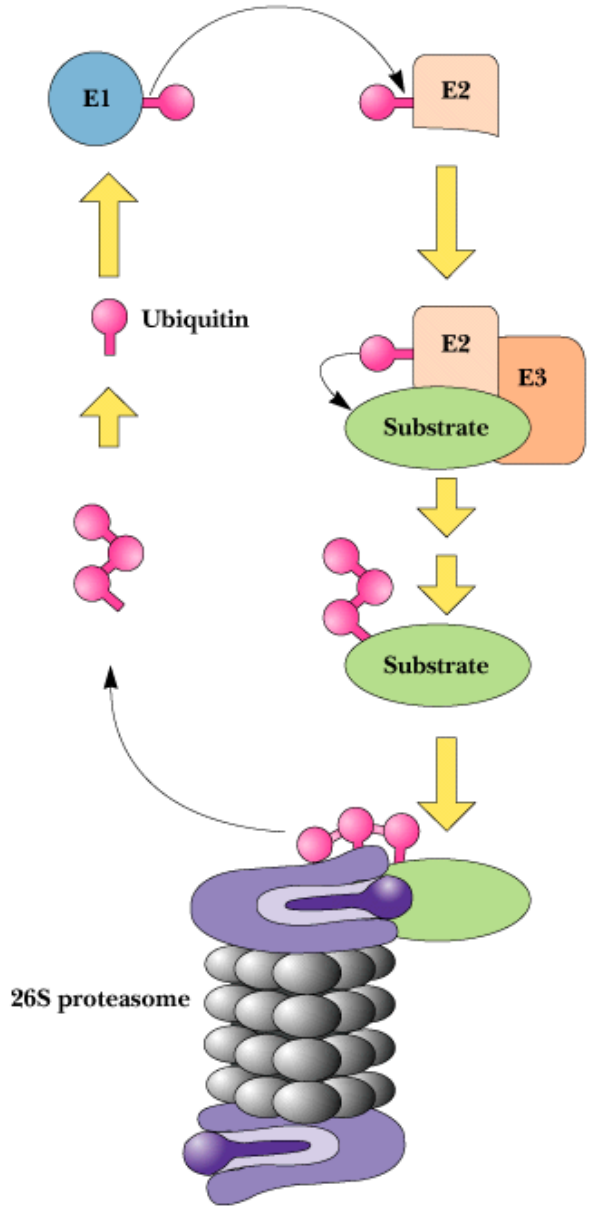
Ubiquitinated Proteins Are Degraded By The 26S Proteosome



- The 26S proteosome is a large protease complex that specifically degrades ubiquinated proteins
- 2 major components – 20S proteosome core, 19S cap.
- Proteolysis occurs in 20S domain
- Ubiquitin recognition occurs at 19S domain

26S Proteosome

- ATP dependent process.
- Protein is unfolded as it enters 20S domain.
- Ubiquitin not degraded, but released and recycled.



Amino Acid Catabolism

1.Deamination

2.Metabolism of Carbon Skeletons

Amino Acids Pool

- The amount of free amino acids distributed throughout the body is called *amino acid pool*
- Plasma level for most amino acids varies widely throughout the day. It ranges between 4-8 mg/dl. It tends to increase in the fed state and tends to decrease in the post absortive state
- Sources of amino acid pool
  - Dietary protein
  - Breakdown of tissue proteins
  - Biosynthesis of nonessential amino acids

Fate of Amino Acid Pool

1. Biosynthesis of structural proteins. Ex: tissue proteins
2. Biosynthesis of functional proteins. Ex : haemoglobin, myoglobin, protein hormones and enzymes
3. Biosynthesis of small peptides of biological importance. Ex: gluthatione, endorphins and enkephalins
4. Biosynthesis of non protein nitrogenous compounds (NPN) as urea, uric acid, creatine, and amomonia
5. Catabolism of amino acids to give ammonia and ot-keto acids

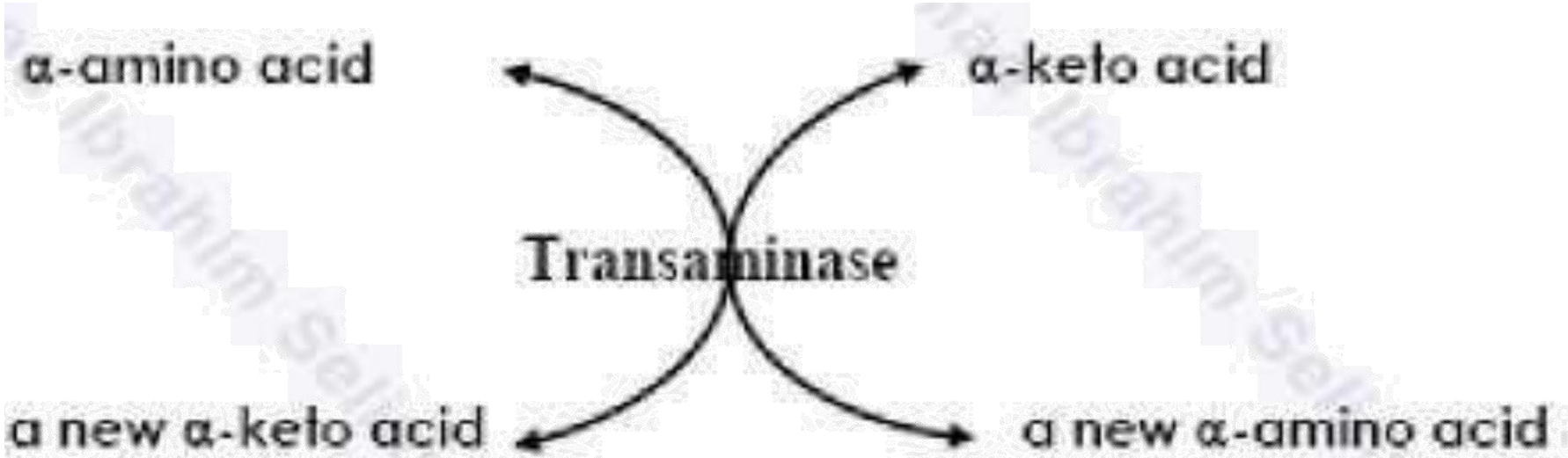
Catabolic Pathways of Amino Acids

1. Transamination
2. Deamination
3. Transamidination
4. Transamidation
5. Decarboxylation

Transamination

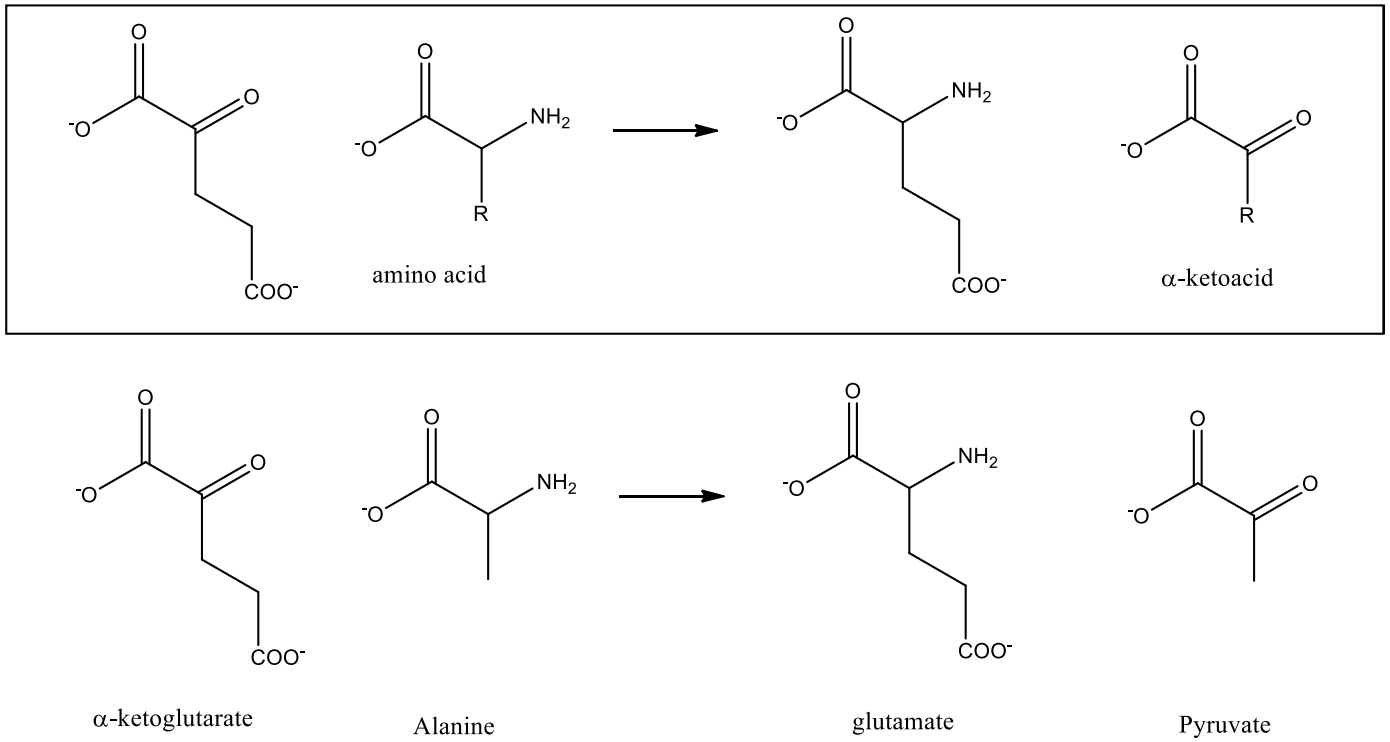
- Transamination means of amino group from α-amino acid to α-keto acid with formation of a new α-amino acid and a new ci-keto acid. The liver is the main site for transamination
- All amino acids can be transminated except lysine, threonine, proline and hydroxyl proline
- All transamination reactions are reversible
- Its catalyzed by aminotransferases (transminases)
- It needs pyridoxal phosphate as a coenzyme

Transamination



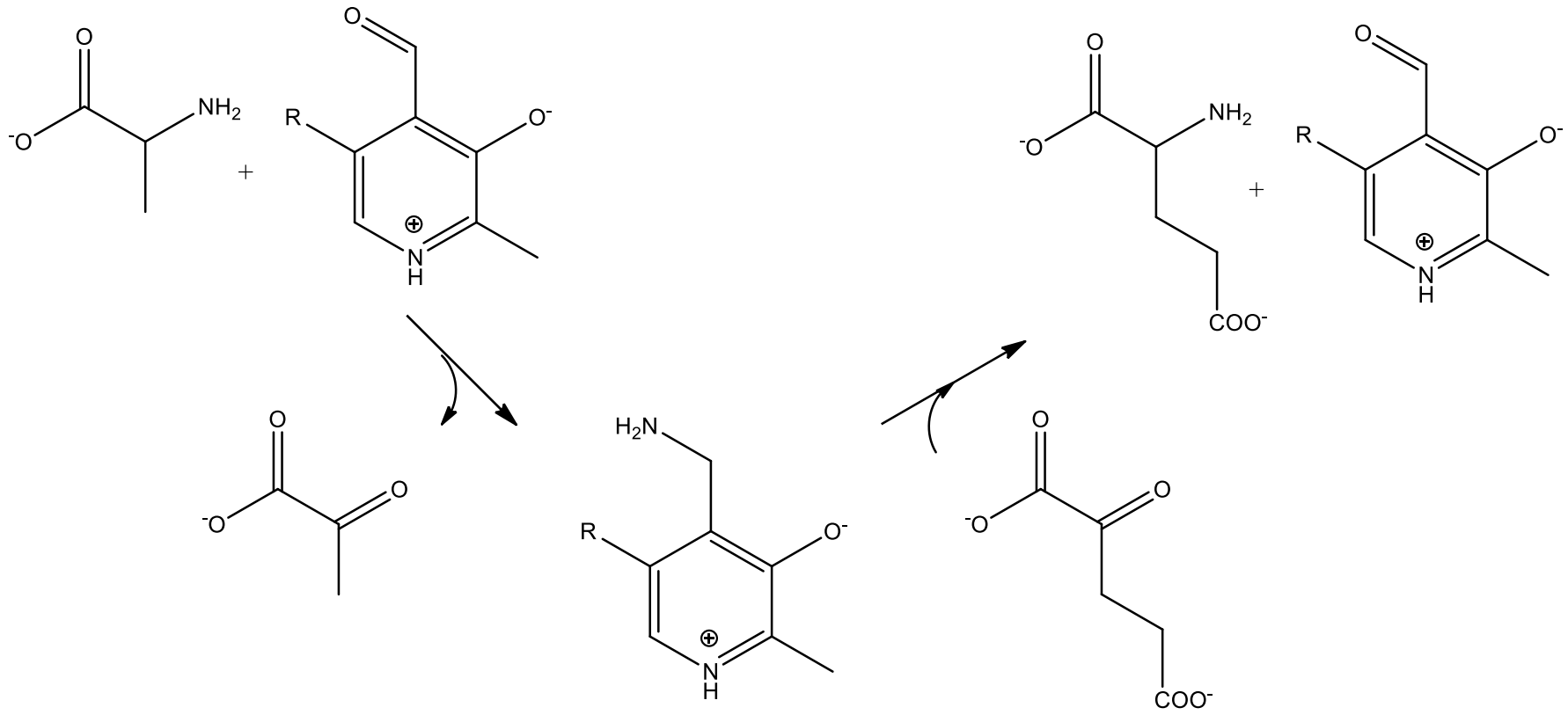
Transamination

All amino acids shuttle their nitrogen toward glutamate



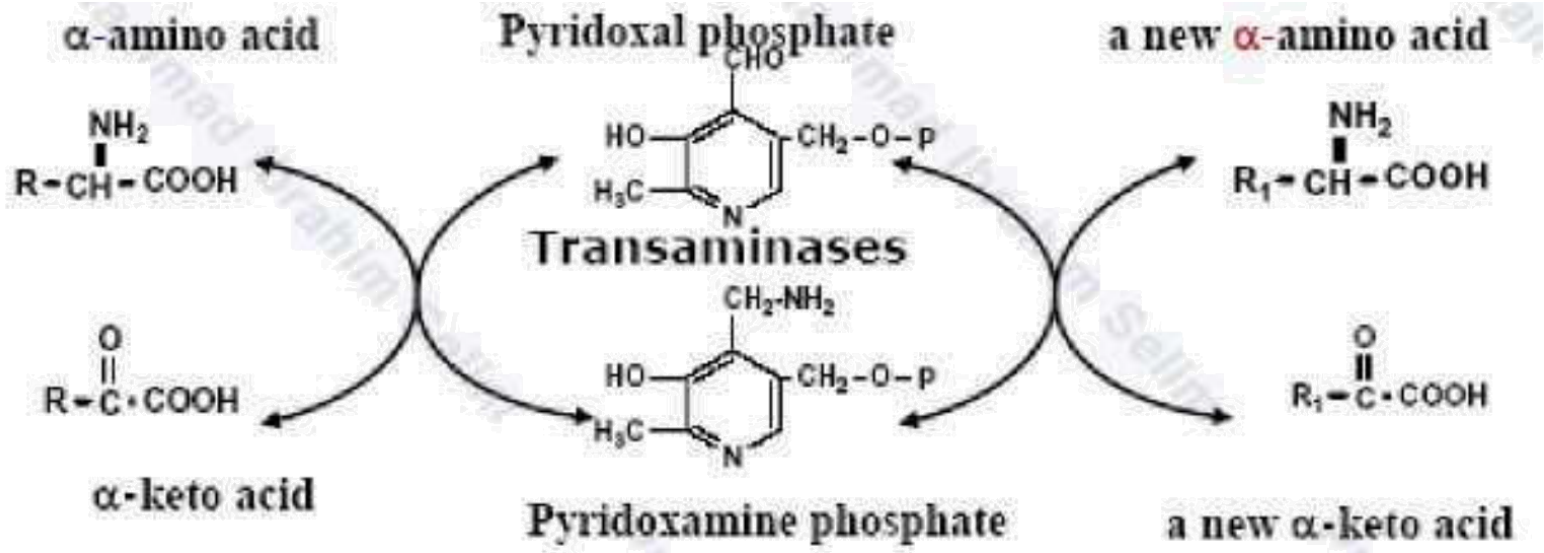
Transamination

- Not an overall Redox reaction
- Requires PLP



Role of Pyridoxal Phosphate in Transamination

- Pyridoxal phosphate acts as an intermediate carrier for amino group
- Pyridoxal phosphate accepts the amino group from amino acid to form pyridoxamine phosphate, which in turn gives the amino group to α-keto acid



Example of Transaminases

1.Alanine transaminase

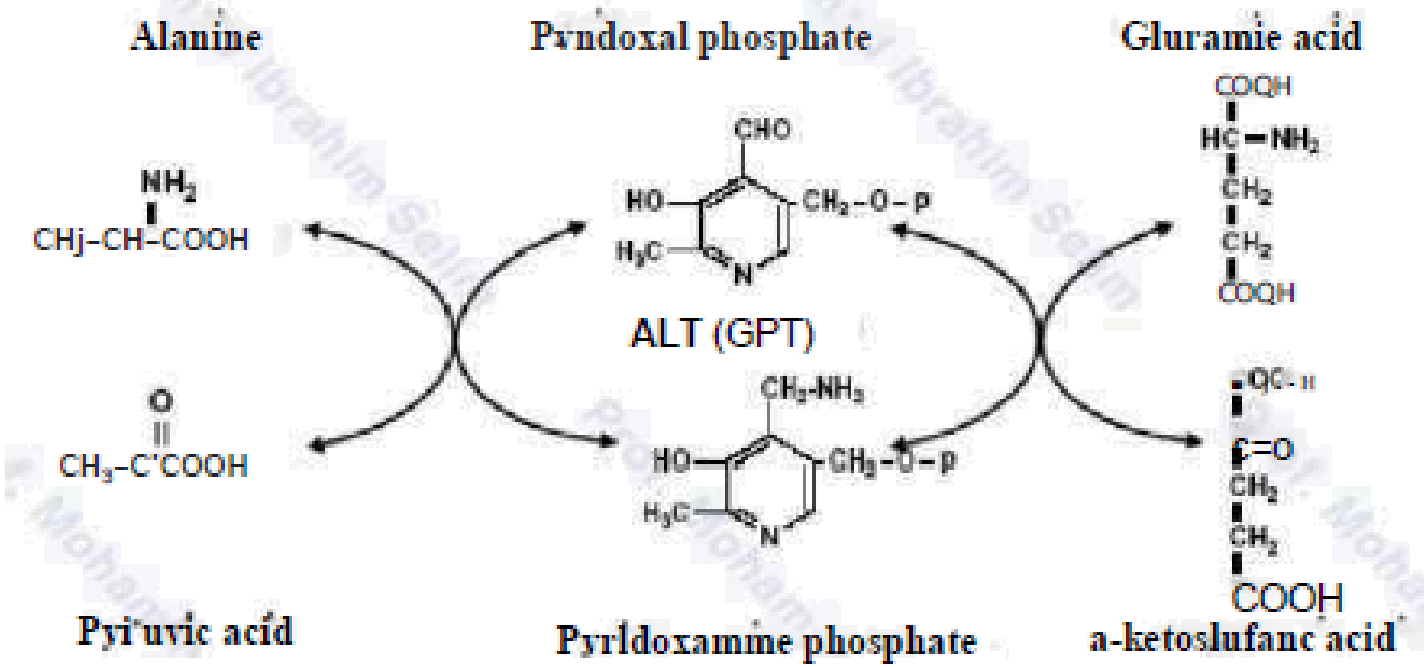
2.Aspartate transaminase

3.Glutamate transaminase

Alanine Transaminase (ALT)

- Its also called Glutamic Pyruvic Transaminase (GPT)
- It catalyzes the transfer of amino group glutamic acid to pyruvic acid to form alanine and ketoglutaric acid
- Its also catalyzes the reverse reaction
- It needs pyridocxal phosphate as a coenzyme
- Its present in cytoplasm of liver cells

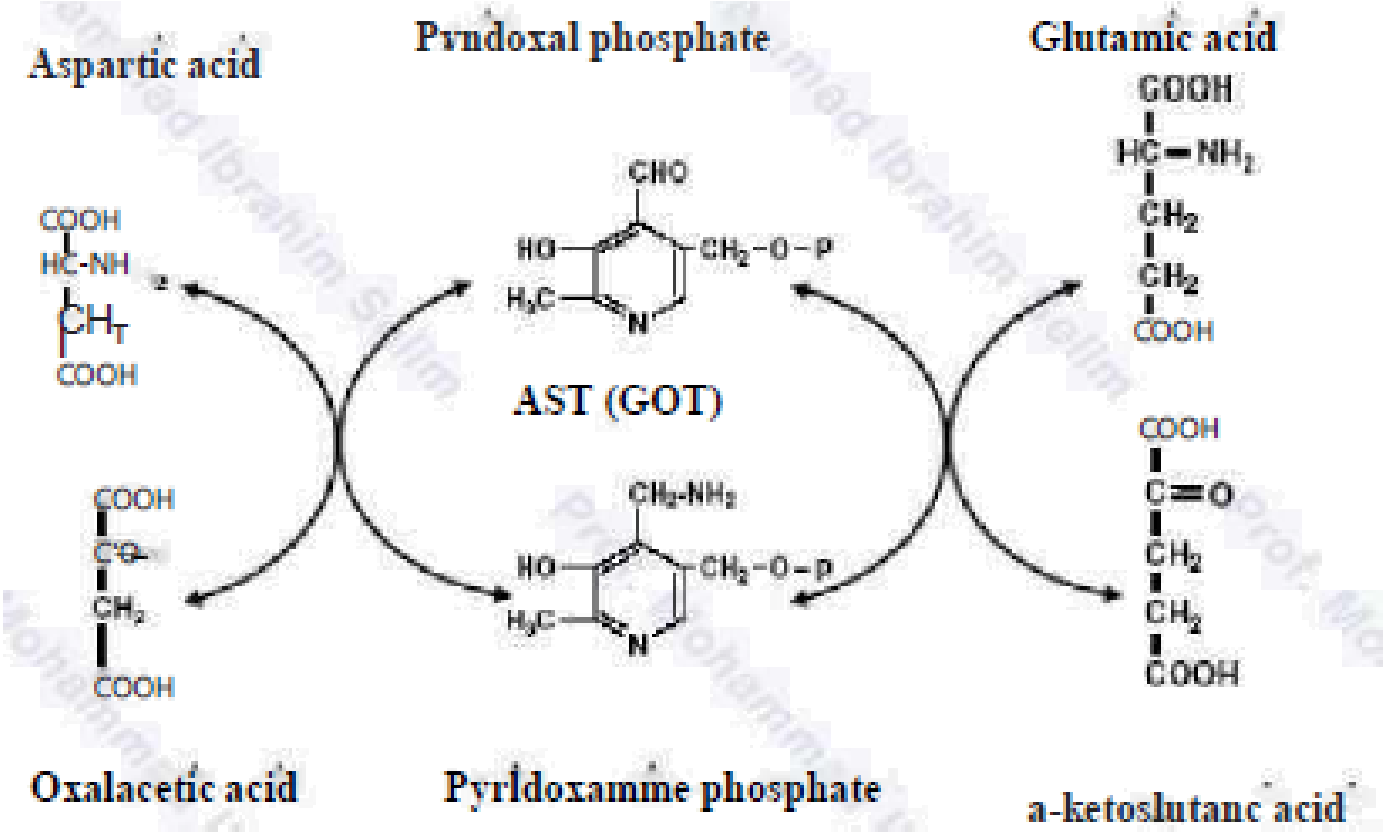
Alanine Transaminase (ALT)



Aspartate Transaminase (AST)

- Its also called Glutamic Oxalacetic Transaminase (GOT)
- It catalyzes the transfer of amino grouo from glutamic acid to form aspartic acid and α-ketoglutaric acid
- It also catalyzes the reverse reaction
- It needs pyridoxal phosphate as a coenzyme
- Its present in liver, heart and skeletal muscle cells
- Its present in both cytoplasm and mitochondria

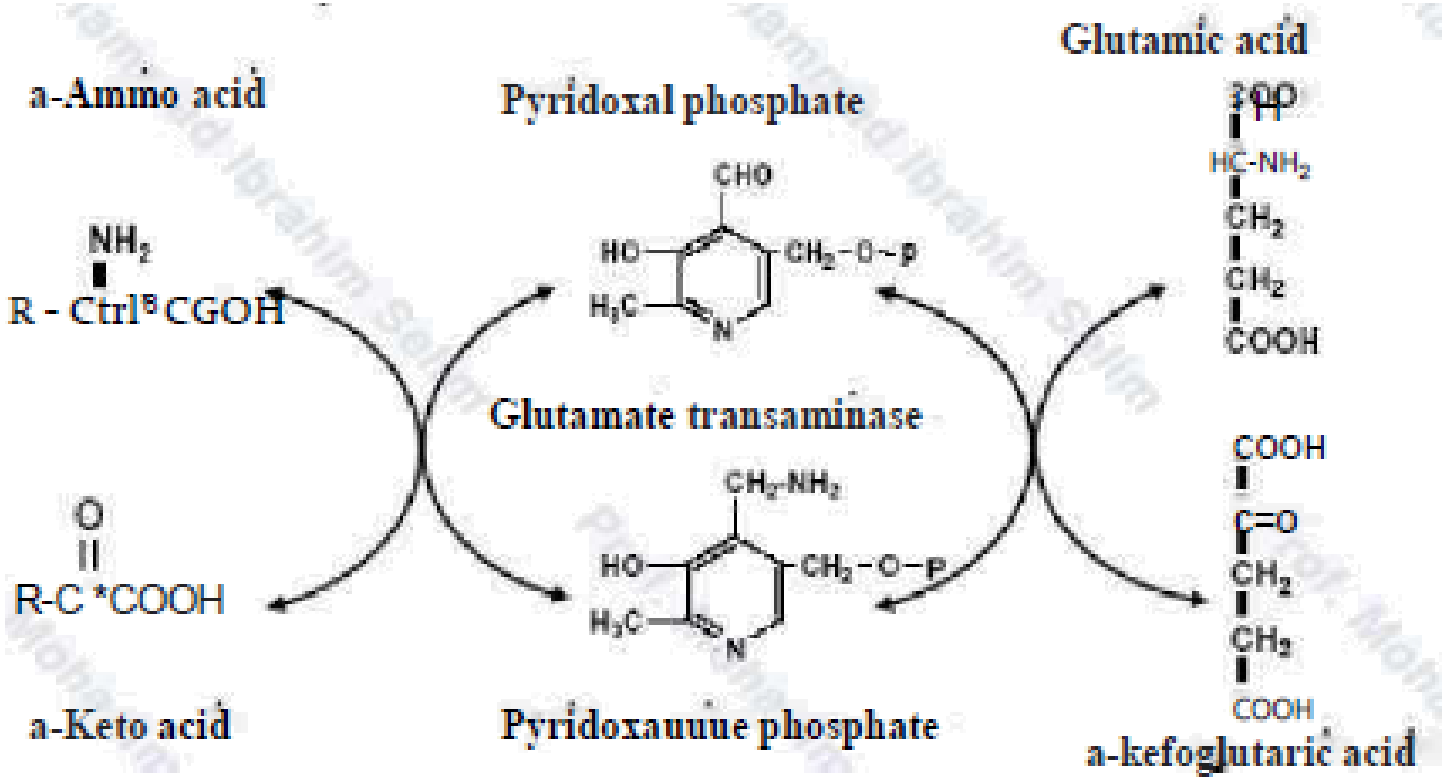
Aspartate Transaminase (AST)



Glutamate Transaminase

- It catalyzes the transfer of amino grouo from any amino acid (except lysine, threonine, proline and hydroxy proline) to α-ketoglutaric acid to form glutamic acid and the corresponding α-keto acid
- It also catalyzes the reverse reaction
- It needs pyridoxal phosphate as a coenzyme
- Its widely distributed in all tissues

Glutamate Transaminase



Clinical Significance of Serum Transaminases

- Transaminases are intracellular enzymes
- Their levels in blood plasma are low under normal conditions
- ALT is present mainly in the cytoplasm of liver cells
- AST is present in both cytoplasm and mitochondria in liver, heart and skeletal muscles
- In liver diseases, there's an increase in both serum ALT and AST levels
- In acute liver diseases, ex: acute viral hepatitis, the increase in more in SGPT in chronic liver diseases, ex: liver cirrhosis the increase is more in SGOT
- In heart diseases, ex: myocardial infarction, there's an increase in SGOT only
- In skeletal muscle diseases, ex:myasthenia gravis, there's an increase in SGOT only

Deamination

Deamination means the removal of amino group from α-amino acid in the form of ammonia with formation of α-keto acid. The liver and kidney are the main sites for deamination. Deamination may be oxidative or non-oxidative :

A. Oxidative deamination

   Its catalyzed by one of the following enzymes :

   - L-amino acid oxidase
   - D-amino acid oxidases
   - Glutamate dehyfrogenase

B. Non-oxidative deamination

   Its catalyzed by one of the following enzymes

   - Dehydratases
   - desulfhydrases

Oxidative Deaminatio

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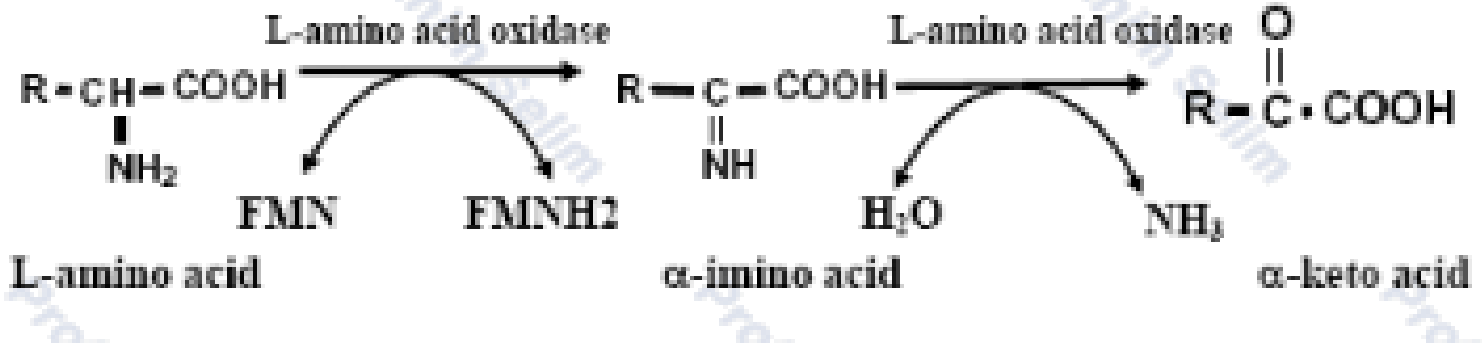
amino acid oxidase

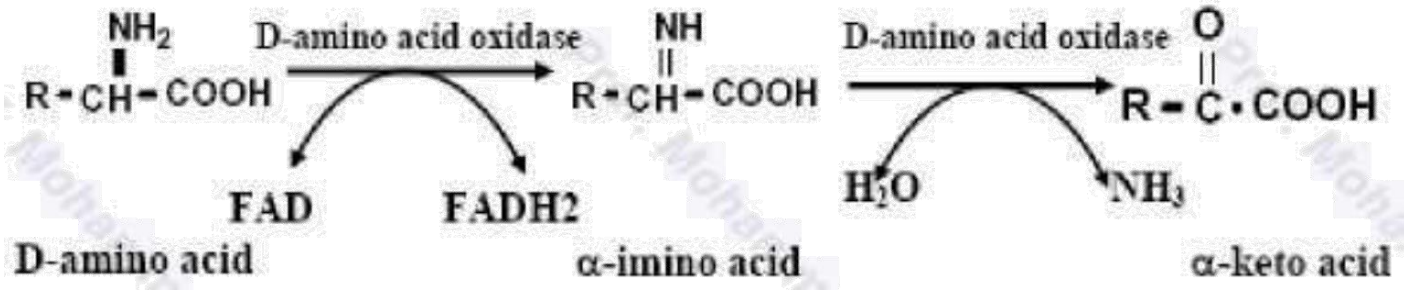
- This enzyme is present in the liver and kidney. Its activity is low
- Its an aerobic dehydrogenase that needs FMN as a coenzyme
- It deaminates most of the naturally occurring L-amino acids



Oxidative Deaminatio n

D-amino acid oxidase

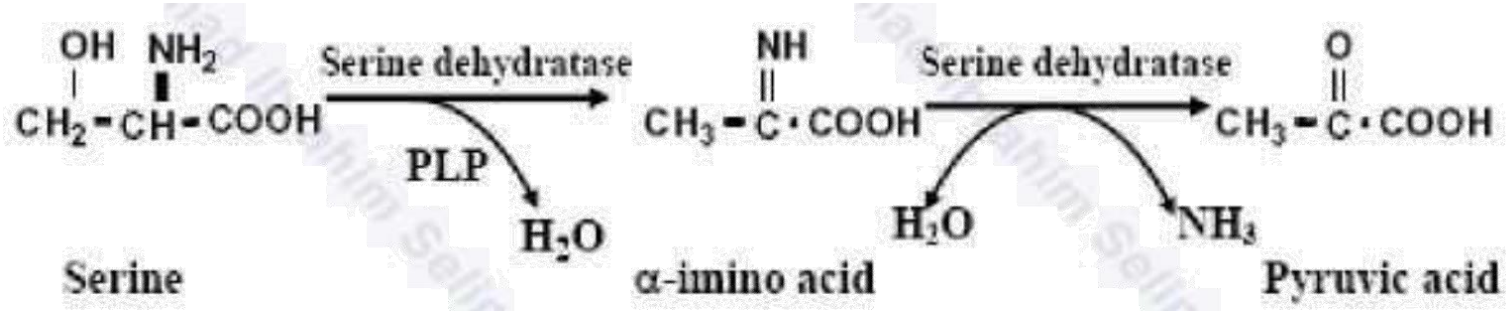
- D-amino acids are present in plants and bacterial cell walls
- They’re not used in protein biosynthesis in human and animals
- D-amino acods are deaminated by D-amino acid oxidase resulting in ammonia and α-keto acid
- D-amino acid oxidase is present in the liver
- Its an aerobic dehydrogenase
- It needs FAD as a coenzyme



Non-Oxidative Deamination

Dehydratase

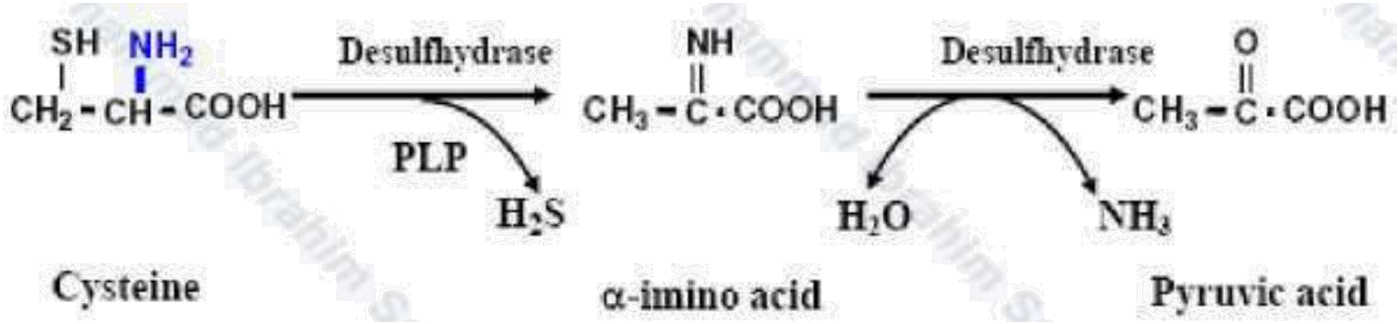
this enzyme deaminates amino acids containing hydroxyl group, ex: serine, homoserine and threonine. It needs pyridoxal phosphate as coenzyme



Non-Oxidative Deamination

Desulfhydrase

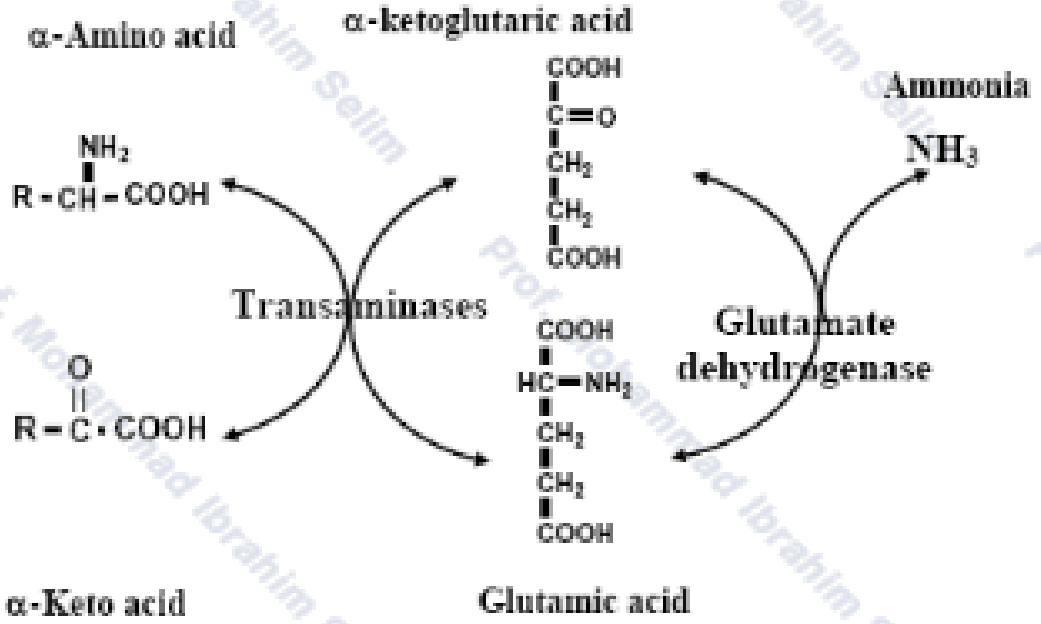
This enzyme deaminates sulpher containing amino acids, ex: cysteine and cysteine. It needs pyridoxal phosphate as a coenzyme



Non-Oxidative Deamination

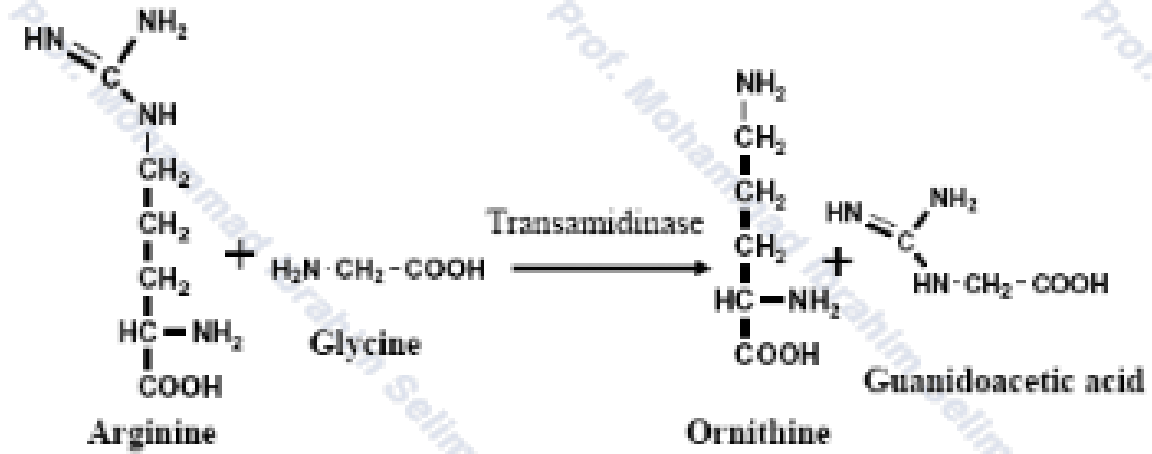
Desulfhydrase

Most of the naturally occurring α-amino acids are catabolized by transamination with α-ketoglutaric acid followed by deamination of the produced glutamic acid, a condition called transdeamination



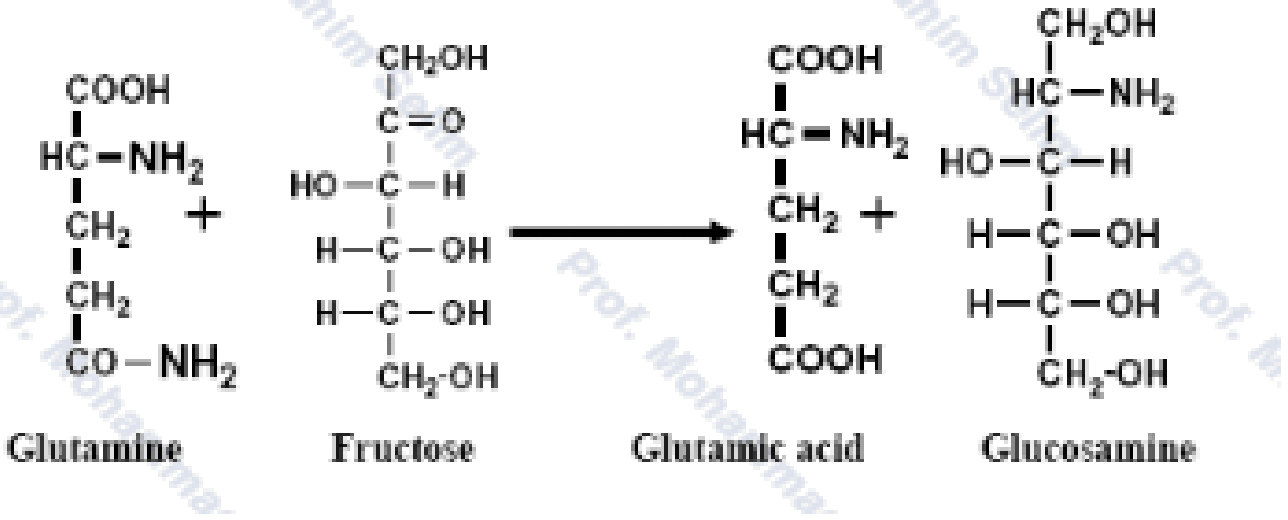
Transamidination

Transamidination means the transfer of amidine group from a donor molecule to an acceptor molecule. its catalyzed by transamidinase enzyme. An example of transmidination reaction is the transfer of amidine group from arginine (donor) to glycine (acceptor) in creatine biosynthesis



Transamidation

Transamidation means transfer of amide group nitrogen from a donor molecule to an acceptor molecule. Its catalyzed by transamidase enzyme. Examples of transamidation reaction include : transfer of amide nitrogen from glutamine (donor) to fructose (acceptor) to form glucosamine; amide group nitrogen of glutamine is the source of N3 and N9 in purine bases



Decarboxylation

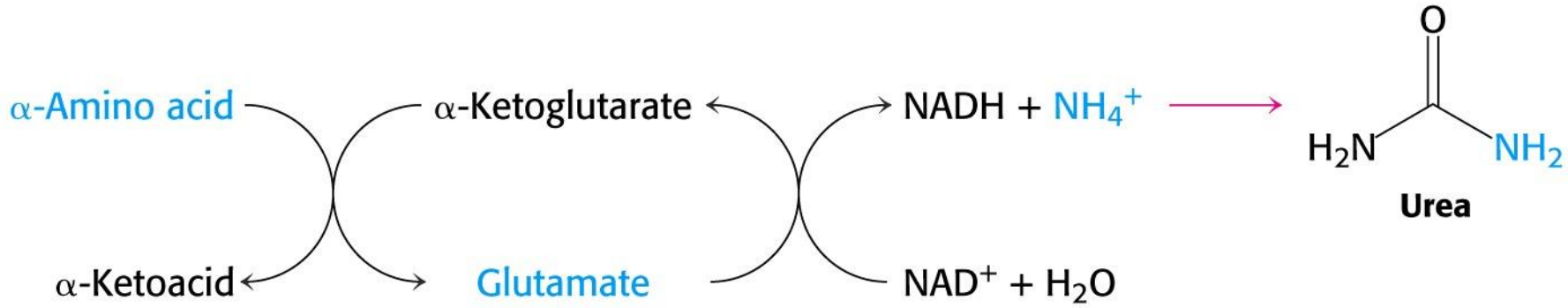
Decarboxylation means removal of CO2 from amino acid with formation of corresponding aminos. Its catalyzed by decarboxylase enzyme. It needs pyridoxal phosphate as a coenzyme. Examples of decarboxylation reaction include : decarboxylation of histidine to form histamine ; decarboxylation of tyrosine to form tyramine

Removal of Nitrogen

Step 1: transamination with a-ketogluturate to form glutamate and new a-keto acid.

Step 2: glutamate is deaminated through oxidative process involving NAD^+

Step 3: form urea through urea cycle.

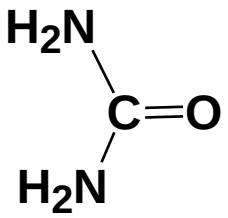


Fate of Ammonia

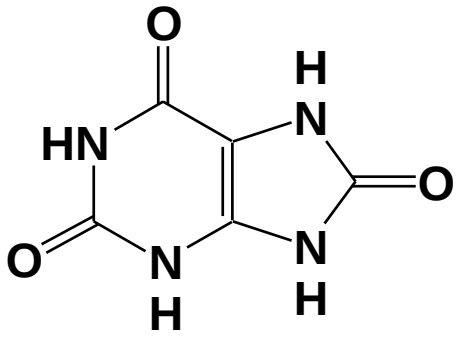
- Ammonia (NH_4^+) is toxic.
- Must not accumulate in cells.
- In humans elevated levels are associated with lethargy and mental retardation
- Mechanism of toxicity unknown.

Mechanism To Get Rid of Ammonia

- Fish excrete ammonia to aqueous environment through gills.
- Birds and reptiles convert ammonia to uric acid and excrete it.
- Mammals convert ammonia to urea in the liver and excrete it in urine.
- Urea is soluble and uncharged, easy to excrete.

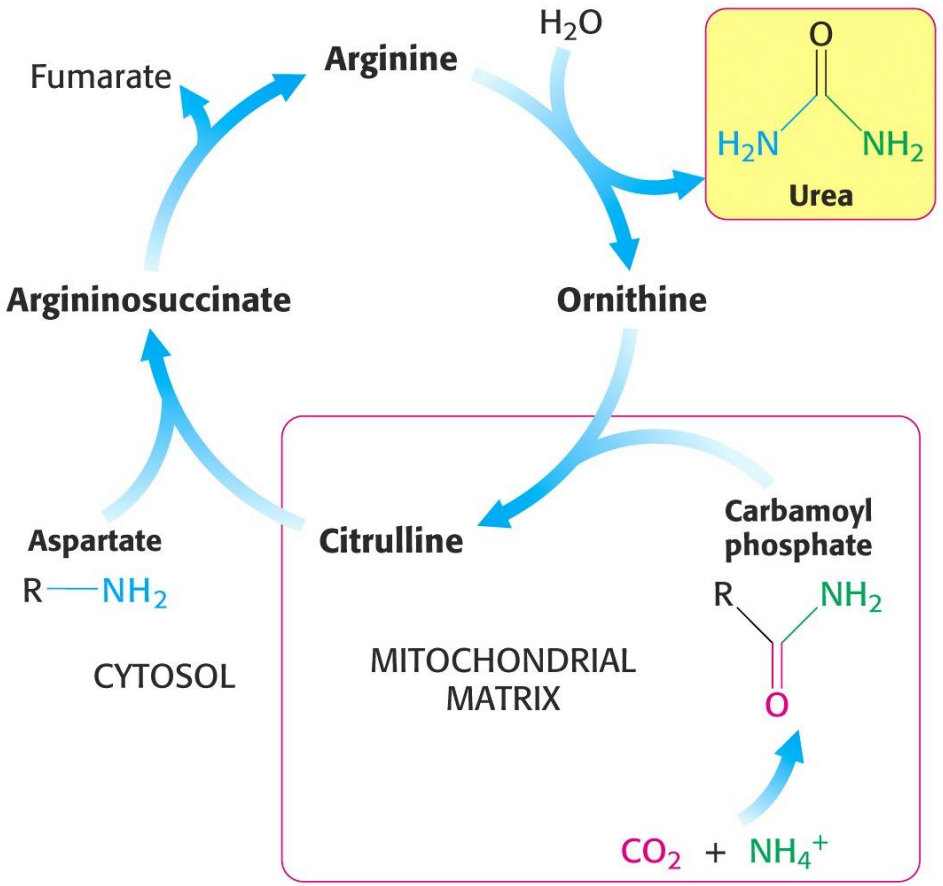


Urea



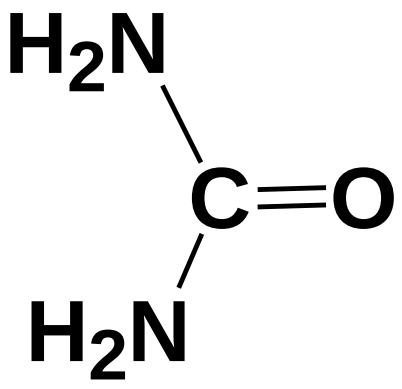
Uric Acid

Urea Cycle


Fumarate
Arginine
H_2O
O
H_2N
NH_2
Urea
Argininosuccinate
Ornithine
Aspartate
R—NH_2
Citrulline
Carbamoyl phosphate
R
NH_2
O
CYTOSOL
MITOCHONDRIAL MATRIX
CO_2 + NH_4^+

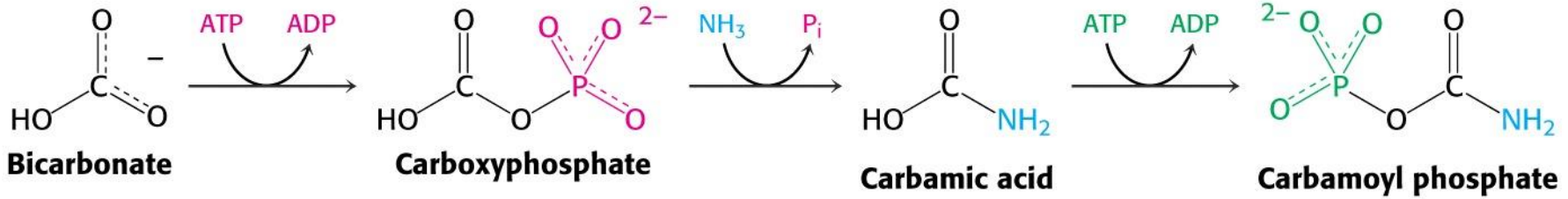
Urea Cycle

- 5 reaction cyclic pathway
- Involves enzymes localized in the mitochondria and cytosol.
- Two amino groups used derived from ammonia and aspartate.
- C an O derived from bicarbonate



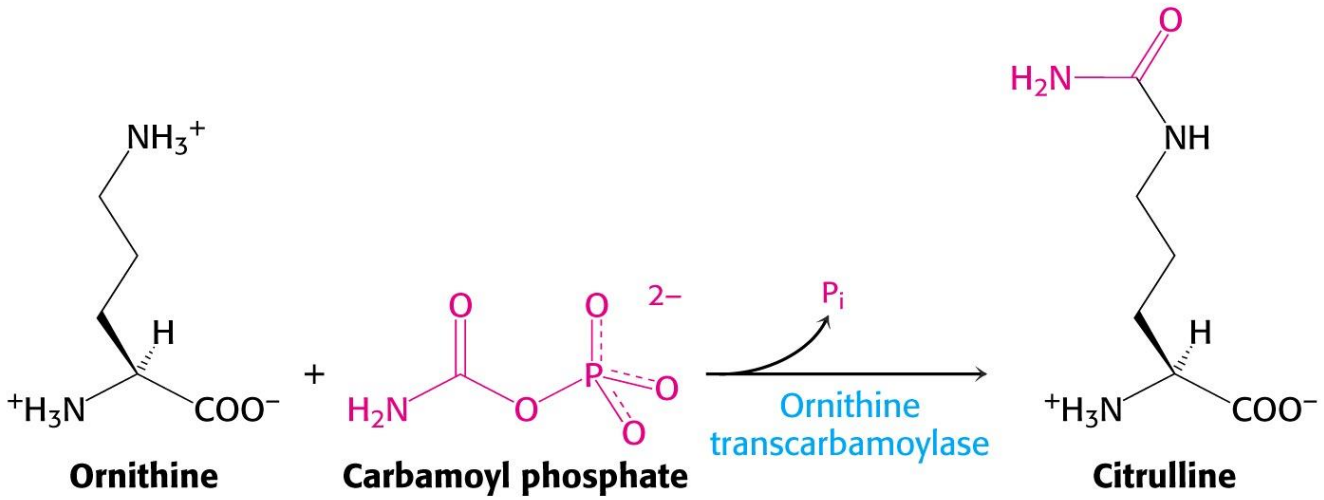
Step 1: Formation of Carbamoyl Phosphate

- Reaction catalyzed by carbamoyl phosphate synthetase I
- Most abundant enzyme in liver mitochondria (makes up 20% of matrix protein)
- Allosterically activated by N-acetylglutamate (acetyl-CoA + glutamate → N-acetylglutamate)
- 2ATP + NH_3 + Bicarbonate →carbamoyl-P + 2ADP



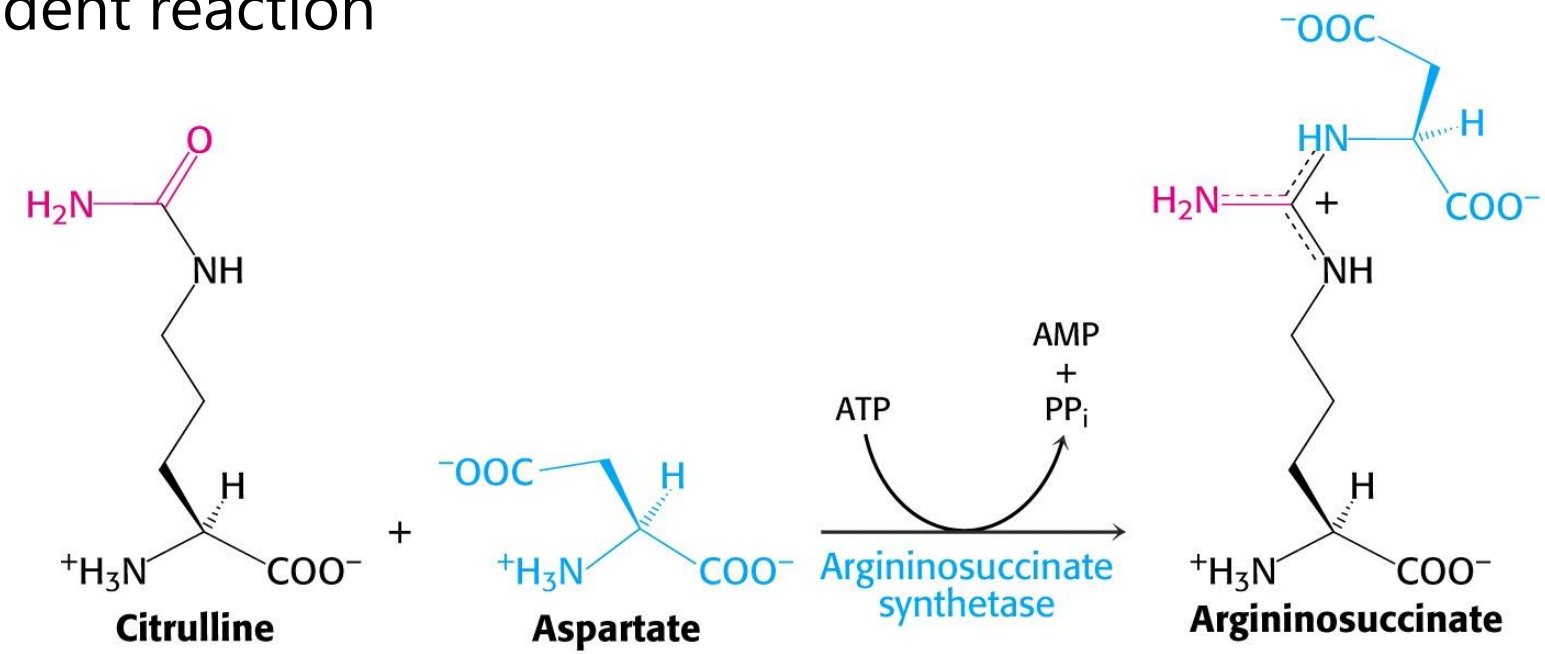
Step 2: Ornithine Transcarbamyolase

- Reaction occurs in mitochondrial matrix.
- Product citrulline is exported out to cytosol



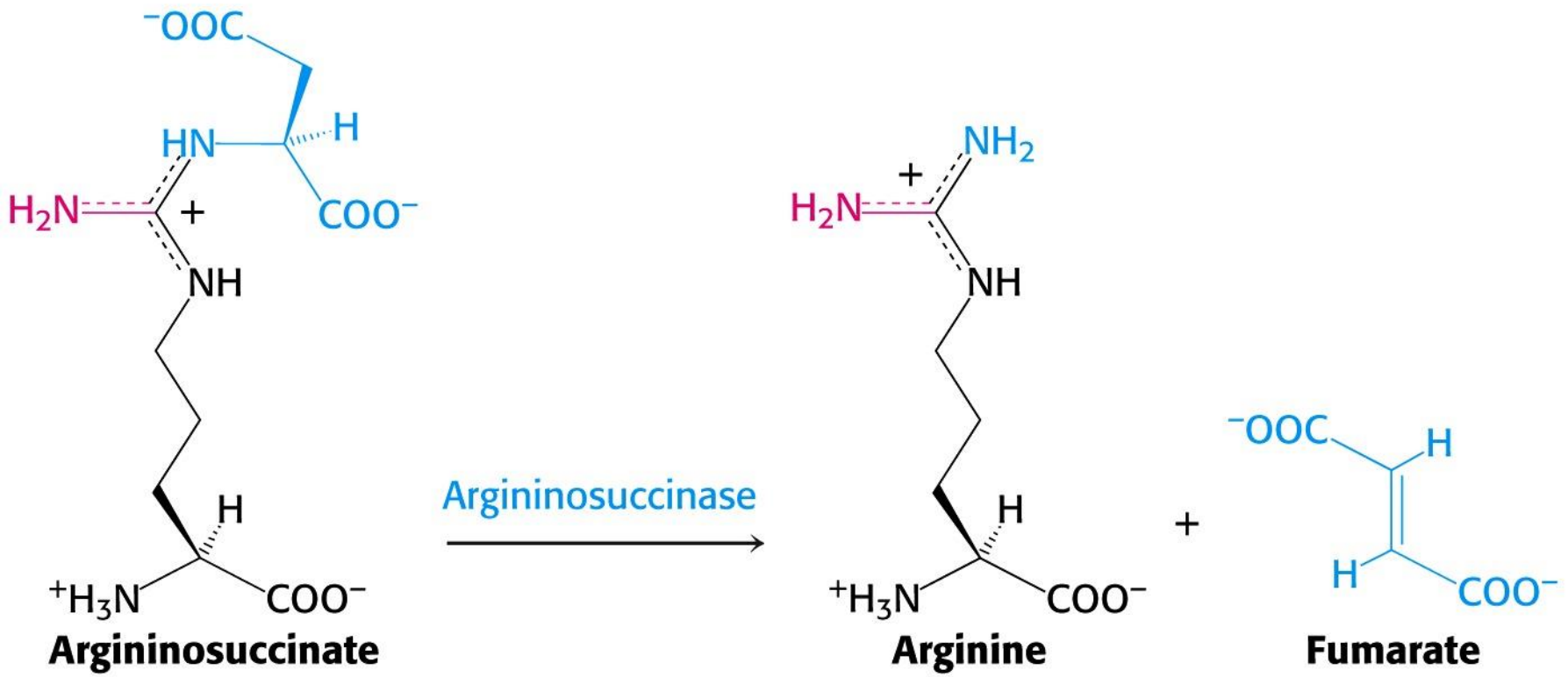
Step 3: Argininosuccinate Synthetase

- Cytosolic enzyme
- 2nd ammonia group incorporates from aspartate
- ATP dependent reaction



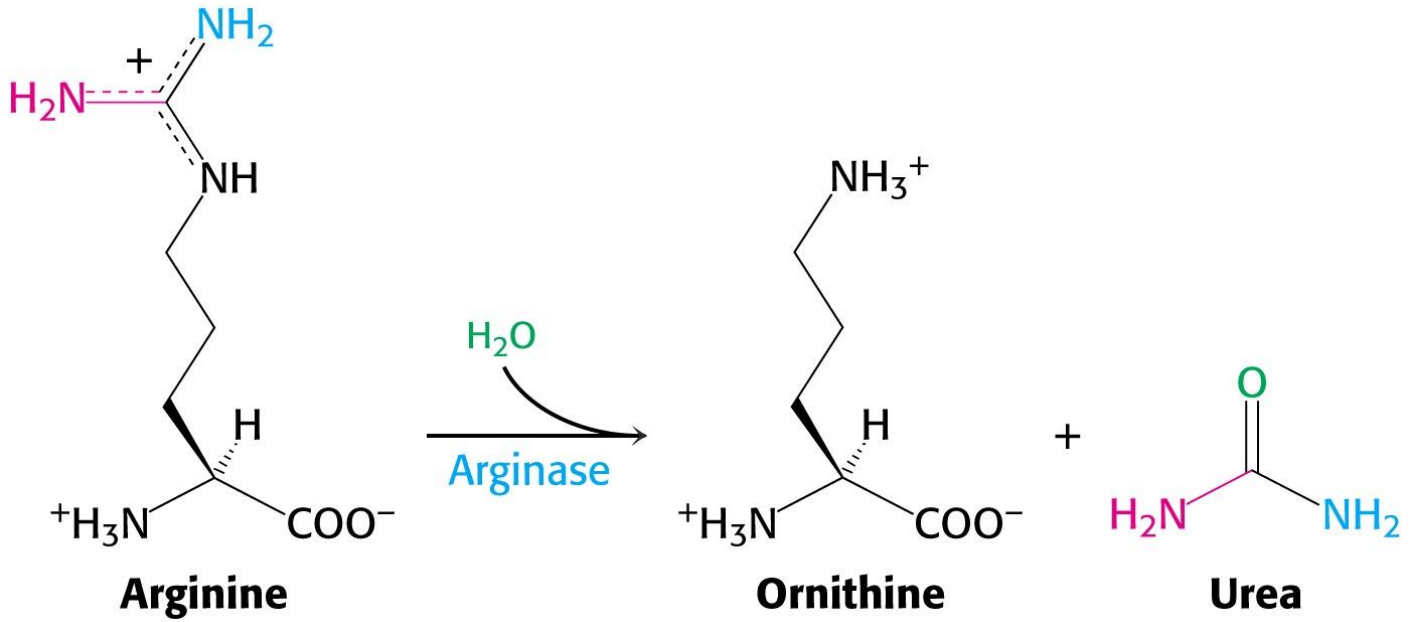
Step 4: Argininosuccinase

- Cytosolic enzyme



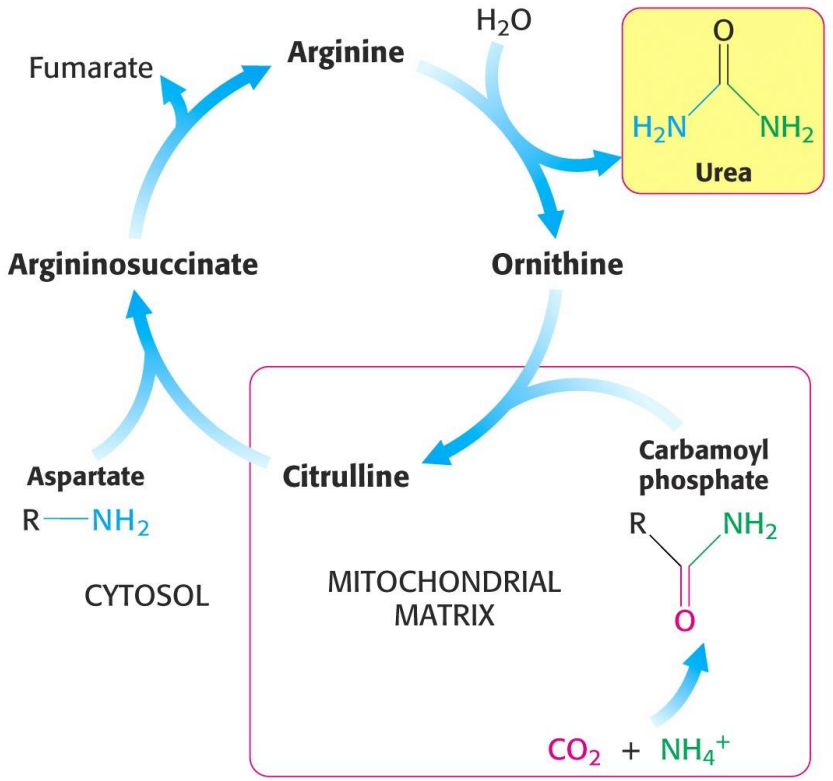
Step 5: Arginase

- Cytosolic enzyme
- Forms urea and ornithine.
- Urea is excreted and ornithine is re-imported into mitochondria



Urea Cycle

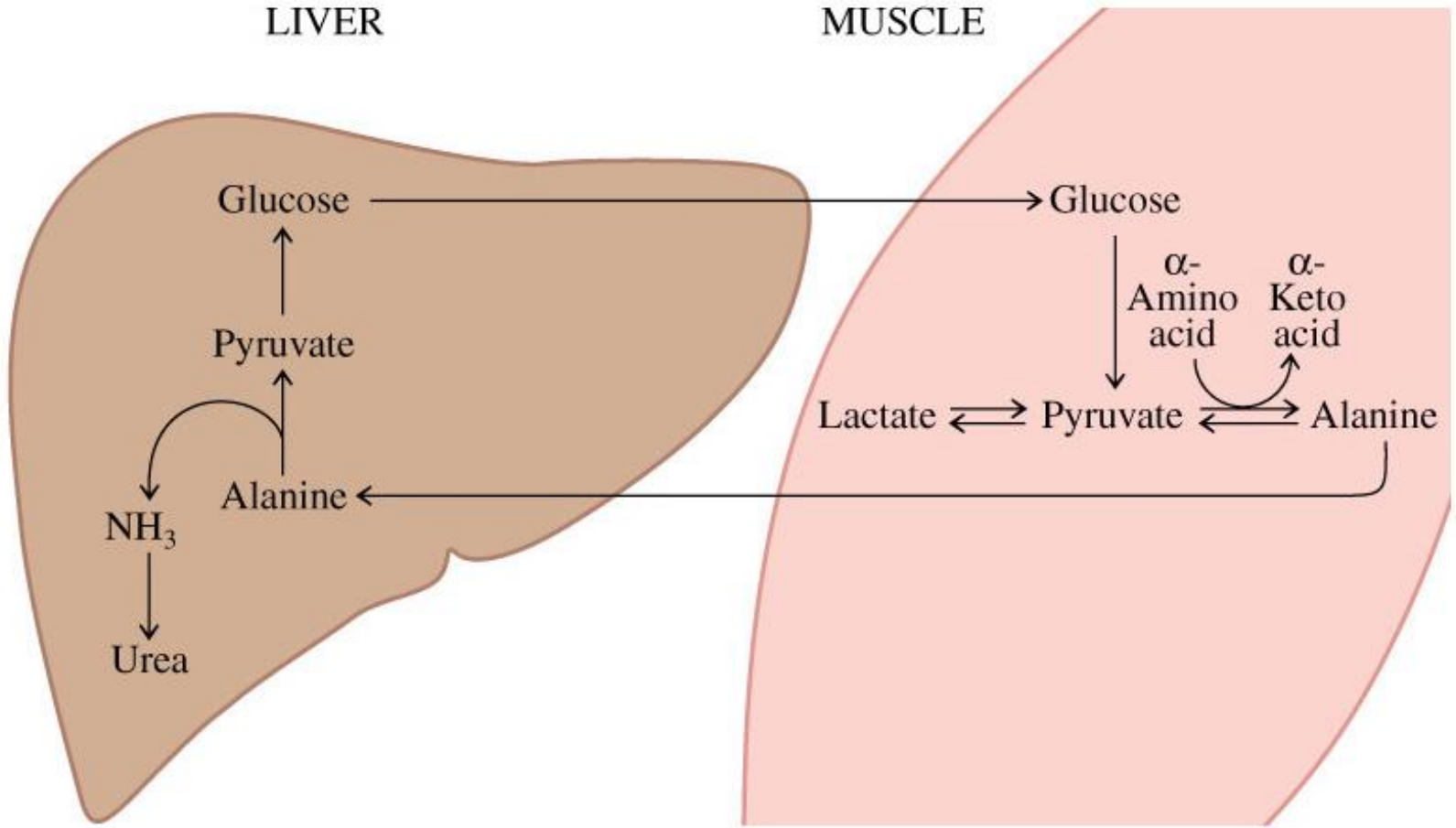
- Requires 3 ATPs + Ammonia + Aspartate + Bicarbonate
- Get urea + fumurate + 2ADP + 2 Pi + AMP + PPi.
- Fumurate skeleton feeds back into TCA



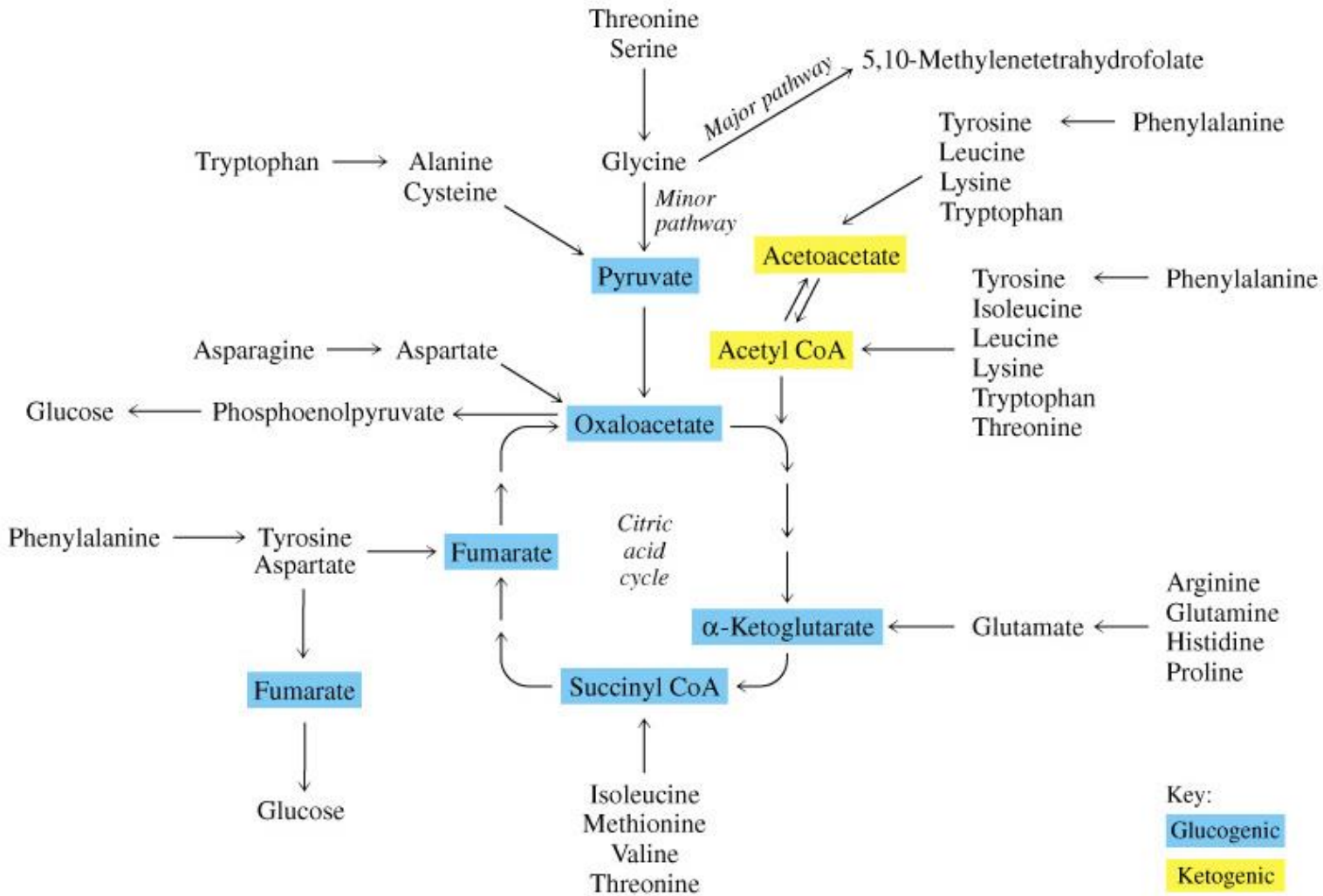
Glucose Alanine Cycle

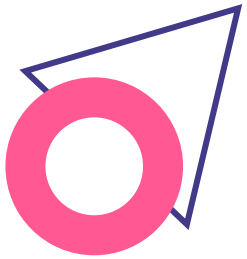
- Amino acid can be catabolized in muscle tissue where carbon skeletons are oxidized for energy.
- Must remove toxic ammonia and transport to liver where it can be converted to urea.
- Amino group from Glu is transferred to pyruvate to form alanine.
- Alanine is exported to the liver via the blood stream where the it is deaminated to pyruvate
- Pyruvate is converted to glucose which is returned to the muscle for fuel.

Glucose Alanine Cycle



Catabolism of Carbon Chains From Amino Acids





Thank You