ZOO 202 : Biophysics & Biochemistry

Gr. B - Unit 6 : Protein Metabolism

(Part 1)

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In animals, amino acids undergo oxidative degradation in 3 different metabolic circumstances:

1. During normal synthesis and degradation of cellular proteins, some amino acids, that are released from protein breakdown are not needed for new protein synthesis, undergo OXIDATIVE DEGRADATION
2. When a diet is rich in protein and the ingested amino acids exceed the body's needs for protein synthesis, the surplus amino acids are catabolized (in the liver amino acids can't be stored)
3. During starvation and uncontrolled DM, when carbohydrates are unavailable or improperly utilized, cellular proteins are used as fuel.

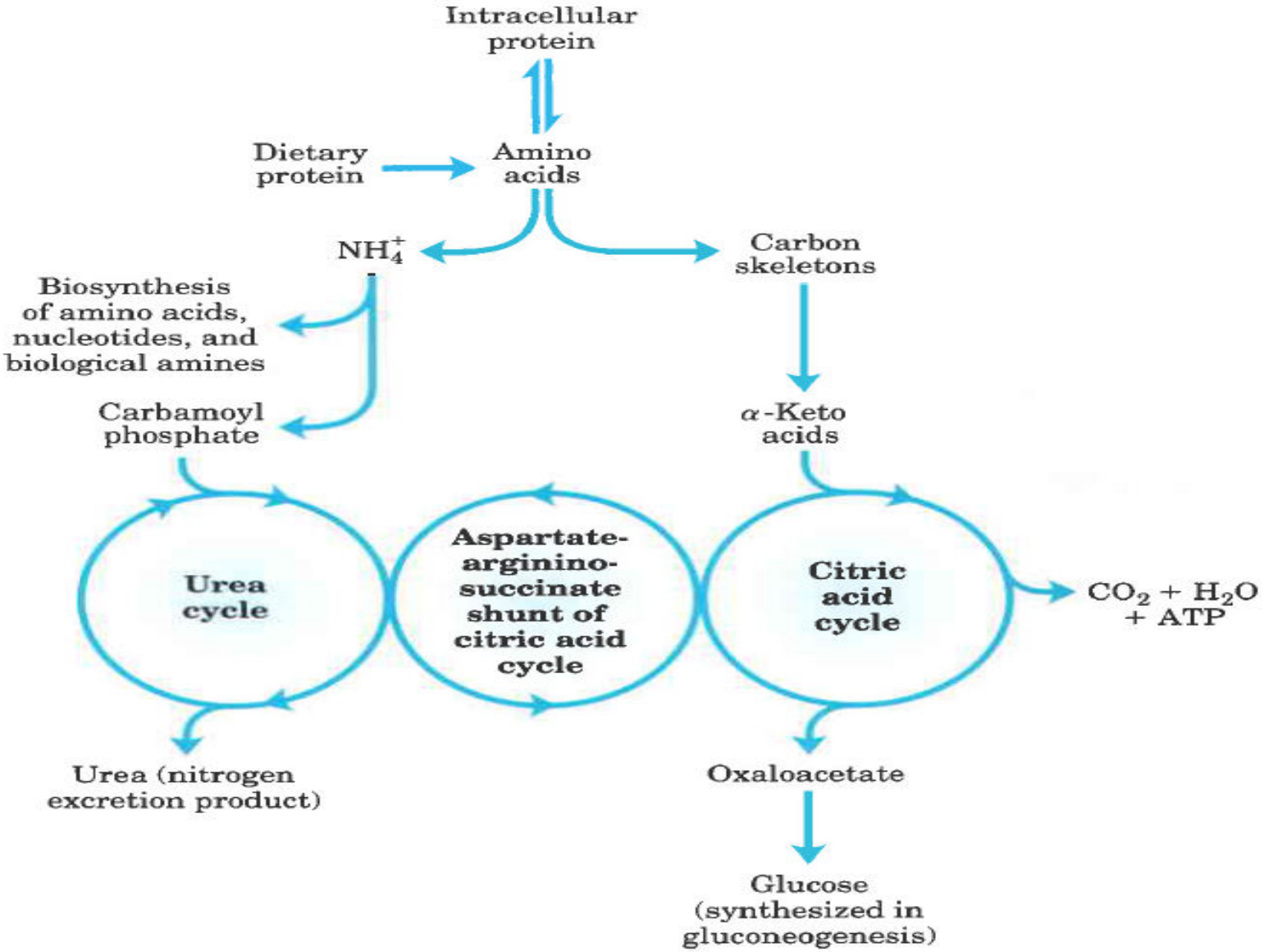


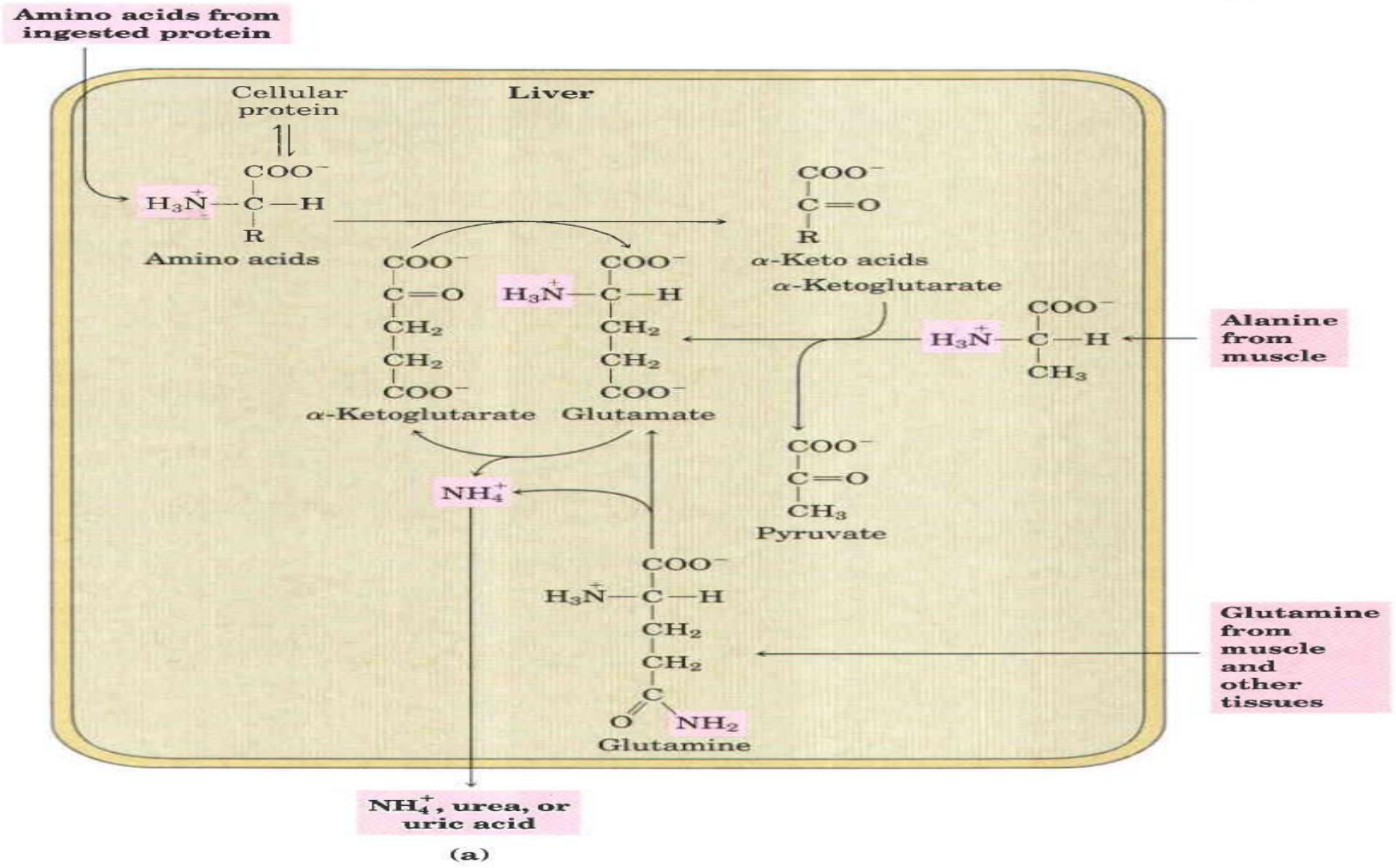
FIGURE 18–1 Overview of amino acid catabolism in mammals. The amino groups and the carbon skeleton take separate but interconnected pathways.

Metabolic fates of amino groups

- Dietary protein are the source of most amino groups
- Most amino acids are metabolized in the liver
- Some of the ammonia generated in this process is recycled and used in a variety of biosynthetic pathways. The excess is either excreted directly or converted to urea or uric acid for excretion, depending on the organism
- Excess ammonia generated in other (extrahepatic) tissues travels to the liver (in the form of amino groups) for conversion to the excretory form.

What happens in Liver?

- Glutamate and glutamine play critical roles in nitrogen metabolism, acting as a kind of general collection point for amino groups.
- In the cytosol of hepatocytes, amino groups from most amino acids are transferred to α-ketoglutarate to form glutamate, which enters mitochondria and gives up its amino group to form NH_4^+ .
- Excess ammonia generated in most other tissues is converted to the amide nitrogen of glutamine, which passes to the liver, then into liver mitochondria.



Overview of catabolism of amino groups in vertebrate liver

In skeletal muscle:

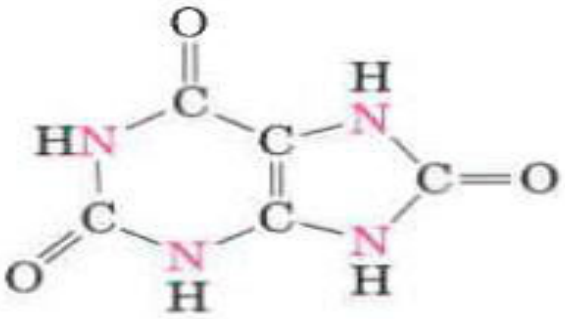
excess amino groups are generally transferred to **pyruvate** to form **alanine**, another important molecule in the transport of amino groups to the liver.

Three excretory products:

NH_4^+

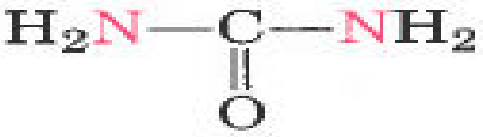
Ammonia (as ammonium ion)

Ammonotelic animals: most aquatic vertebrates, such as bony fishes and the larvae of amphibia



Uric acid

Uricotelic animals: birds, reptiles



Urea

Ureotelic animals: many terrestrial vertebrates; also sharks

AMMONOTELISM

- It is the type of excretion in which ammonia is the main nitrogenous waste material. Such animals are called ammonotelic.
- Ammonia is produced as a result of catabolism of proteins, especially in the liver cells by oxidative deamination of excess of amino acids in the presence of OXIDASE enzyme.
- **Occurrence**

  It is found in aquatic animal groups like sponges, coelentrates, crustaceans, echinoderms, bony fish, tadpole larvae and salamander

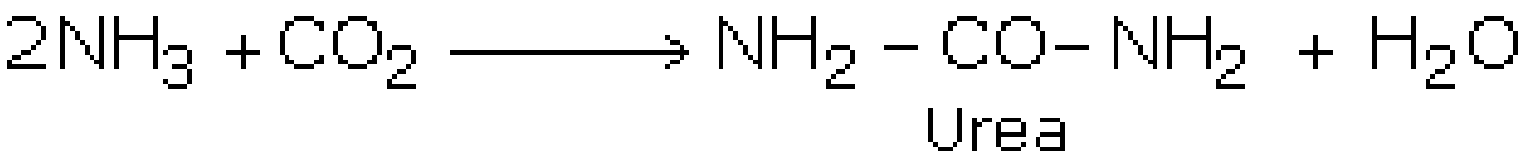
Ammonia is highly toxic and must be metabolised or expelled from the body as soon as possible

- Ammonia is highly soluble in water and a very large volume of water is needed by the animal to dissolve ammonia.
- 1 gm of ammonia needs about 300 - 500 ml of water.
- But this is not a problem for animals living in an aqueous habitat which are generally found to be ammonotelic

UREOTELISM

- It is a type of excretion where urea is the main nitrogenous waste material. Animals showing ureotelism are called ureotelic animals
- **Occurrence**

  Generally found in land animals which can afford to excrete sufficient volume of water or to concentrate urea in considerable quantity in the urine. It is commonly found in man, whales, seals, desert mammals like kangaroo rats, camels, toads, frogs, cartilagenous fishes, aquatic and semi aquatic reptiles like alligator, terrapins and turtles.

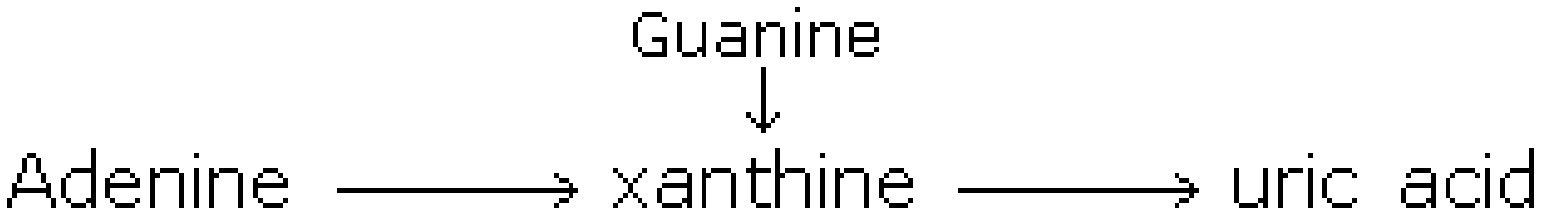


- In the liver of the animals, ammonia is detoxified to form urea by the ornithine cycle.
- Urea is far less toxic than ammonia and so can remain inside the body for a longer period without causing any ill effects inside the body
- 1 gm of urea needs about 50 ml of water to be expelled out.

URICOTELISM

- Elimination of uric acid as the main nitrogenous waste material is called uricotelism. Animals showing uricotelism are called uricotelic animals.
- **Occurrence**

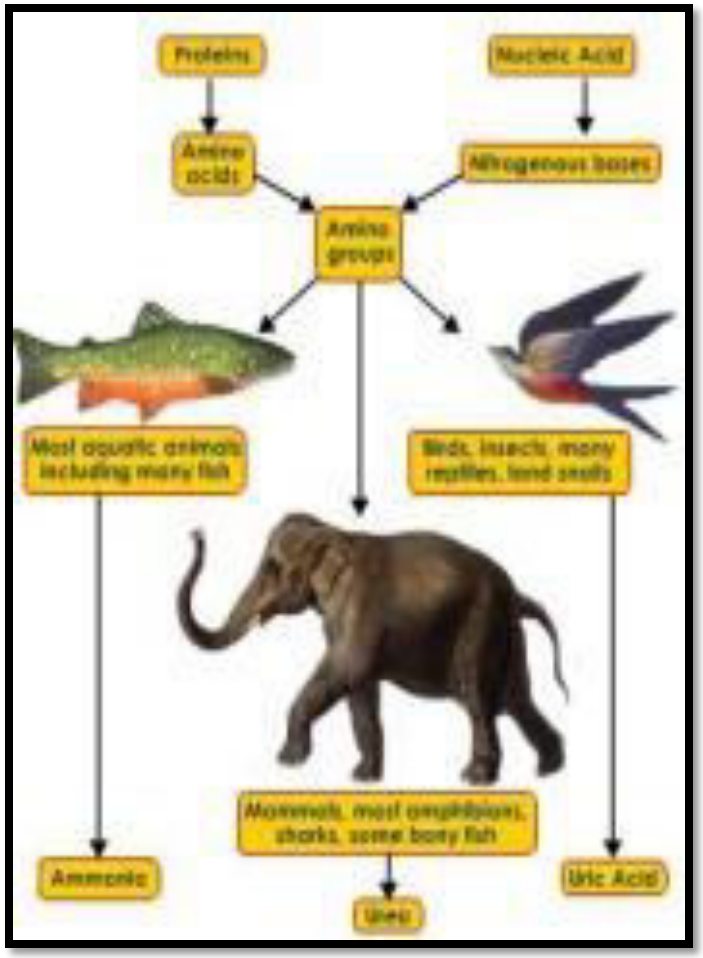
  It is a common method seen in birds, land reptiles, insects, land snails and some land crustaceans.



- Uric acid is formed from ammonia mostly in the **liver** and to some extent in the **kidneys**.
- The process is highly energy dependant, but is **much less toxic than both ammonia and urea** and it is almost **insoluble in water** and can be eliminated from the body in nearly a solid state, **saving a lot of water**.

How uric acids are eliminated?

- Since kidneys can handle the nitrogenous wastes only in solution, reptiles and birds pass a dilute solution of uric acid into the **CLOACA**, where water is absorbed and **solid uric acid is eliminated along with faeces**.
- The faecal matter of certain birds like cormorants, pelicans and gannets called **guano** has been used for the commercial extraction of uric acid. Islands off the coast of South America are covered with guano
- Man also excretes a small amount of uric acid in his urine formed by the catabolism of nucleic acids.



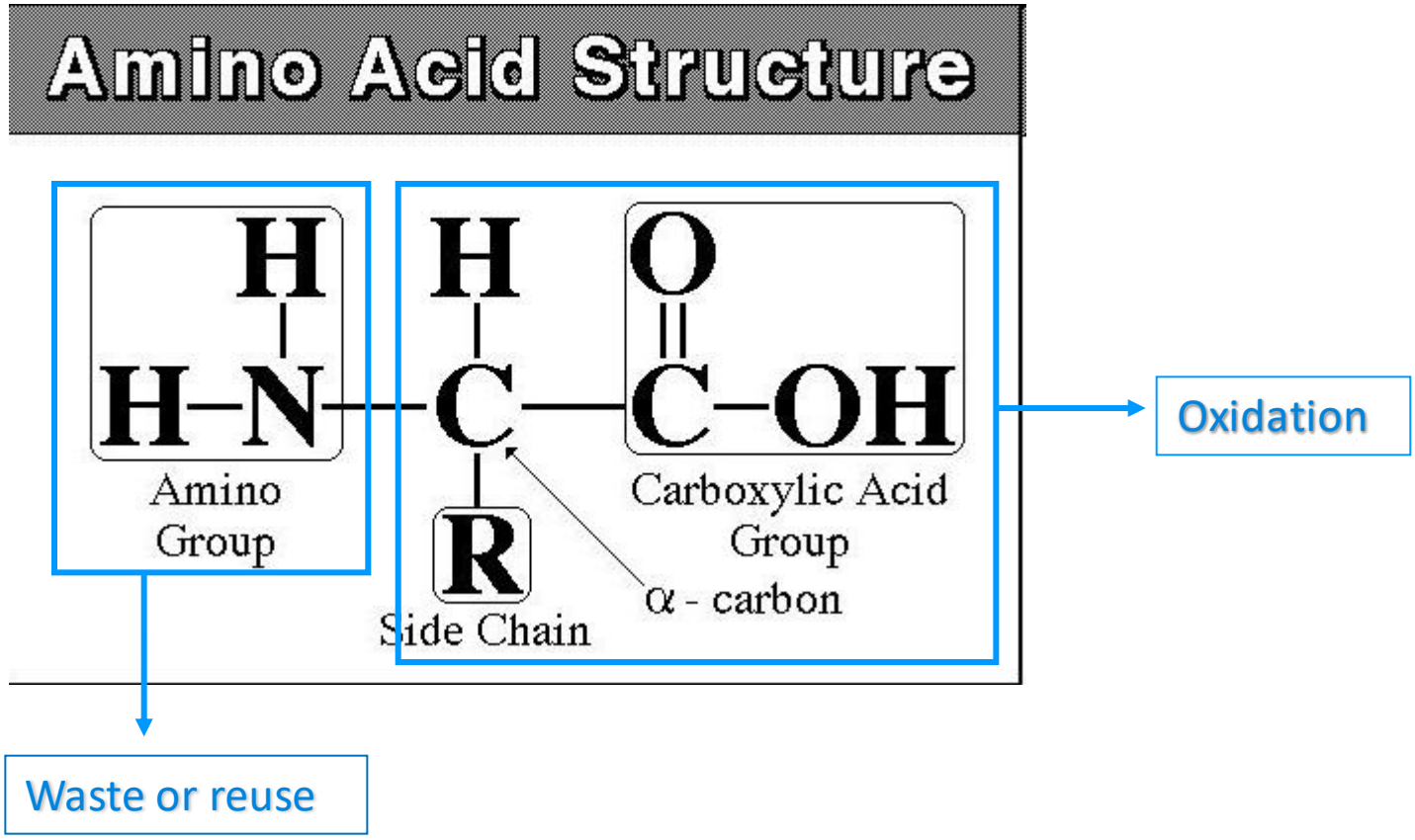
Ammonotelism

Uricotelism

Ureotelism

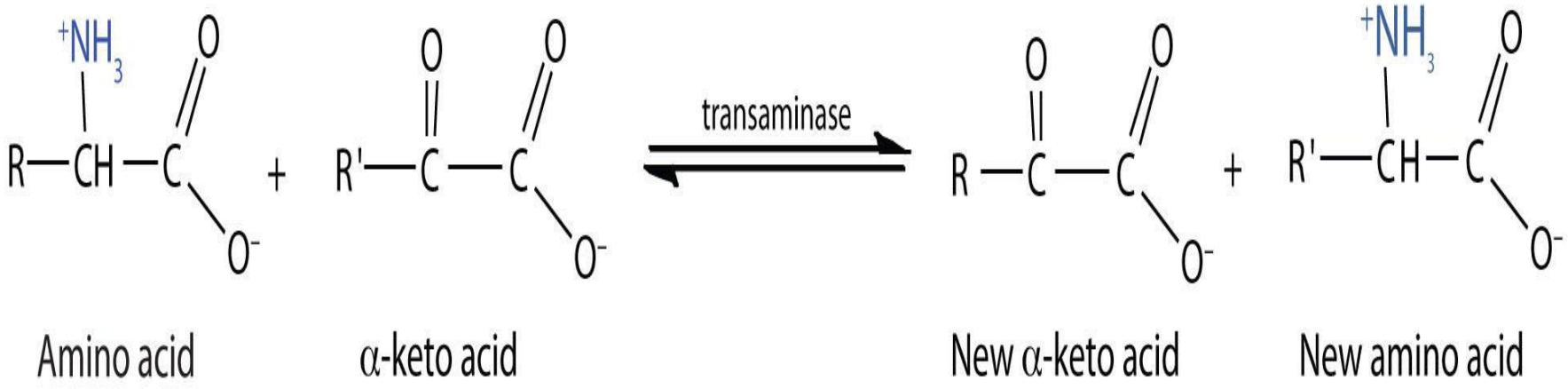
Ammonotelism vs Ureotelism vs Uricotelism

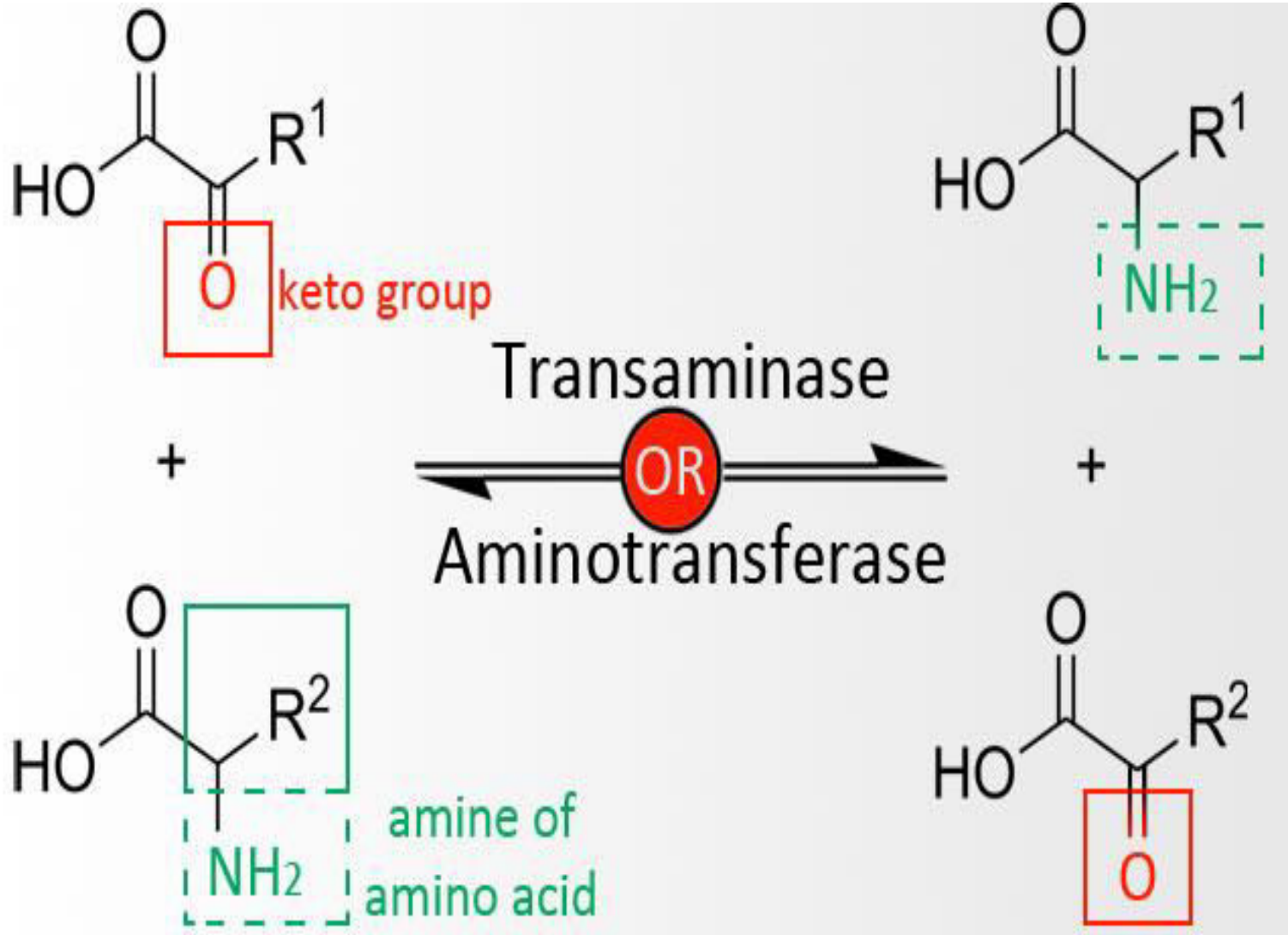
Types/ Characters	Ammonotelism	Ureotelism	Uricotelism
Chief Nitrogenous waste	Ammonia	Urea	Uric acid
Solubility	Easily dissolves in liver cells	Less soluble in water	Almost insoluble in water
Origin	Deamination of amino acids in liver cells	Ornithine cycle in liver	Potassium ureates reacts with water & Co_2 to form uric acid
Toxicity	Very toxic	Less toxic	Very low toxicity


Amino Acid Structure
H
H–N
Amino
Group
H
C
R
Side Chain
O
C–OH
Carboxylic Acid
Group
α - carbon
Oxidation
Waste or reuse

TRANSAMINATION

- A process in which the amino group of an amino acid is transferred to a keto acid so that latter changes to a new amino acid while the original amino acid converts into a new keto acid.




O
HO
R1
O
keto group
+
Transaminase
OR
Aminotransferase
+
O
HO
R1
NH2
O
HO
R2
NH2
amine of
amino acid
O
HO
R2
O

Features:

- Catalyzed by **TRANSAMINASE** or **Aminotranferase**
- Acts on **L-amino acids** only
- It involves **deamination** and **amination** side by side without liberating free ammonia
- Require **Pyridoxal phosphate** as cofactor
- **Pyridoxamine** is the intermediate in the reaction
- Transamination interconverts pairs of α-amino acids and α-ketoacids.
- Reversible process
- Aminotransferases remove the amino group from most amino acids and produce the corresponding α -ketoacid

Occurrence:

Mainly in LIVER

KIDNEY, BRAIN, HEART, TESTIS

- Amino acids that don't participate in transamination:

  Basic a.a - Lysine, Hydroxy a.a - threonine, Heterocyclic a.a - proline, hydroxyproline.

EXAMPLES:

- Alanine-pyruvate amino transferase (alanine aminotransferase) and glutamate α-ketoglutarate amino transferase (glutamate aminotransferase) catalyze the transfer of amino groups to pyruvate (forming alanine) or to α-ketoglutare (forming glutamate)

- Each aminotransferase is specific for one pair of substrates but nonspecific for the other.
- Since alanine is also a substrate for glutamate aminotransferase, all the amino nitrogen from amino acids that undergo transamination can be concentrated in glutamate

- The effect of transamination reaction is to collect the amino groups from many different amino acids in the form of L-glutamate.
- L-glutamate then functions as an amino group donor for biosynthetic pathways or for excretion pathways that lead to the eliminaton of nitrogenous waste product's.

- Glutamate releases its amino group as ammonia in the liver.
- In hepatocytes, glutamate is transported from cytosol into mitochondria, where it undergoes OXIDATIVE DEAMINATION by glutamate dehydrogenase.

Mechanism of Transamination

Step – I Enzyme binds to its cofactor Pyridoxal phosphate (PLP)

Step – II Amino acid (1) binds to PLP linked enzyme. Enzyme - Schiff base I is formed, water goes out.

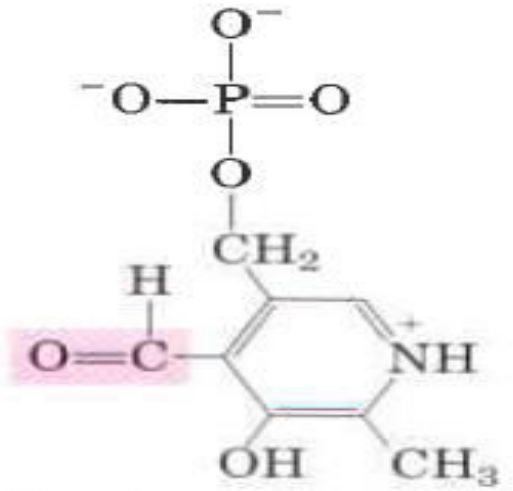
Step – III Tautomerization of Schiff base I to Schiff base II (Aldemine to Ketimine)

Step – IV Schiff base II reacts with water. Formation of Keto acid (1) and Enzyme-PMP

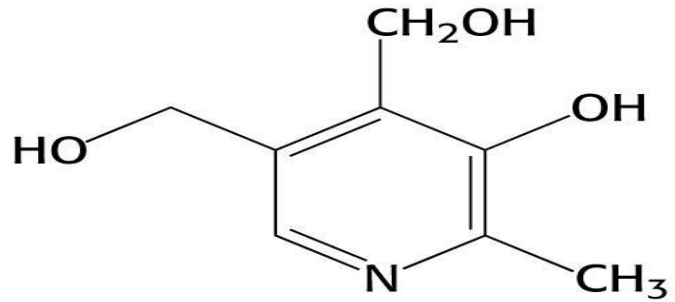
Mechanism of Transamination

- Step – V Next Keto acid (2)combines with Enzyme-PMP. Enzyme Schiff base II' is formed and water goes out
- Step – VI Schiff base II' tautomerize to Schiff base I' (Ketimine to Aldemine form)
- Step – VII Schiff base I' reacts with water and dissociates into Amino acid (2) and Enzyme-PLP

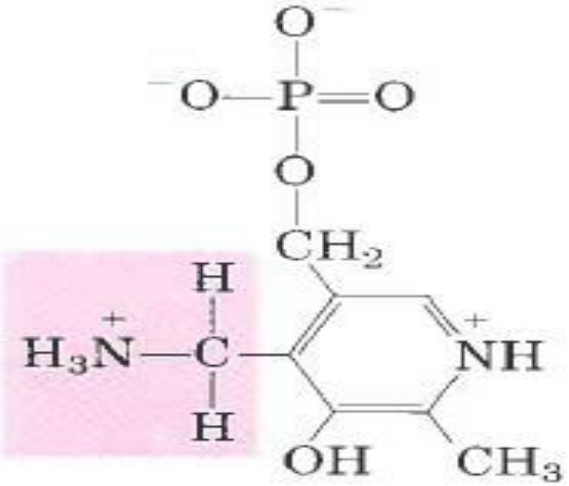
Structure of Pyridoxine, PLP & PMP



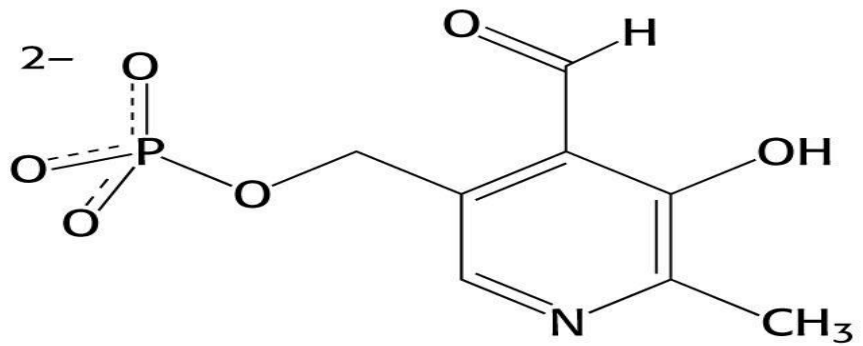
Pyridoxal phosphate (PLP)



Pyridoxine (Vitamin B_6)



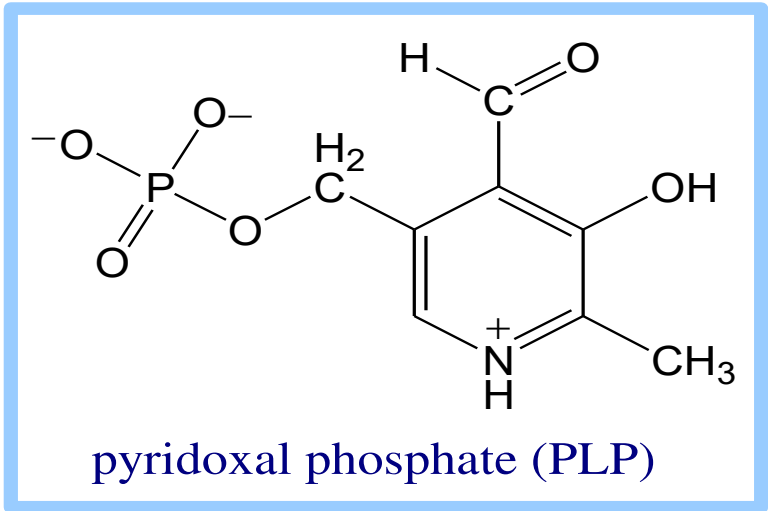
Pyridoxamine phosphate



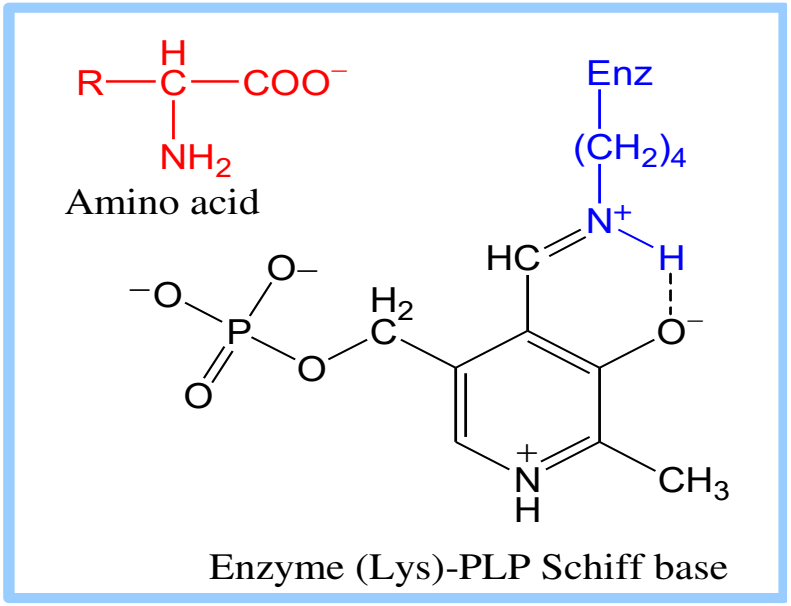
Pyridoxal phosphate (PLP)

Enzyme binds to its co-factor PLP.

- In the resting state, the aldehyde group of pyridoxal phosphate is in a **Schiff base** linkage to the ε-amino group of an enzyme **lysine** side-chain.

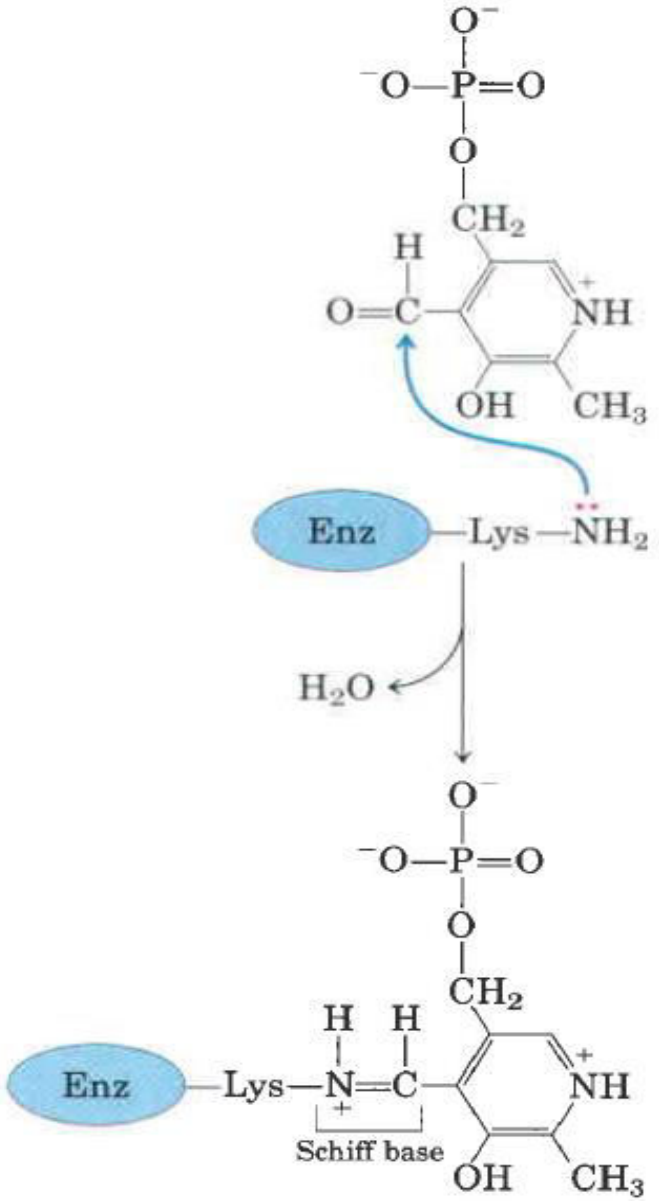


pyridoxal phosphate (PLP)

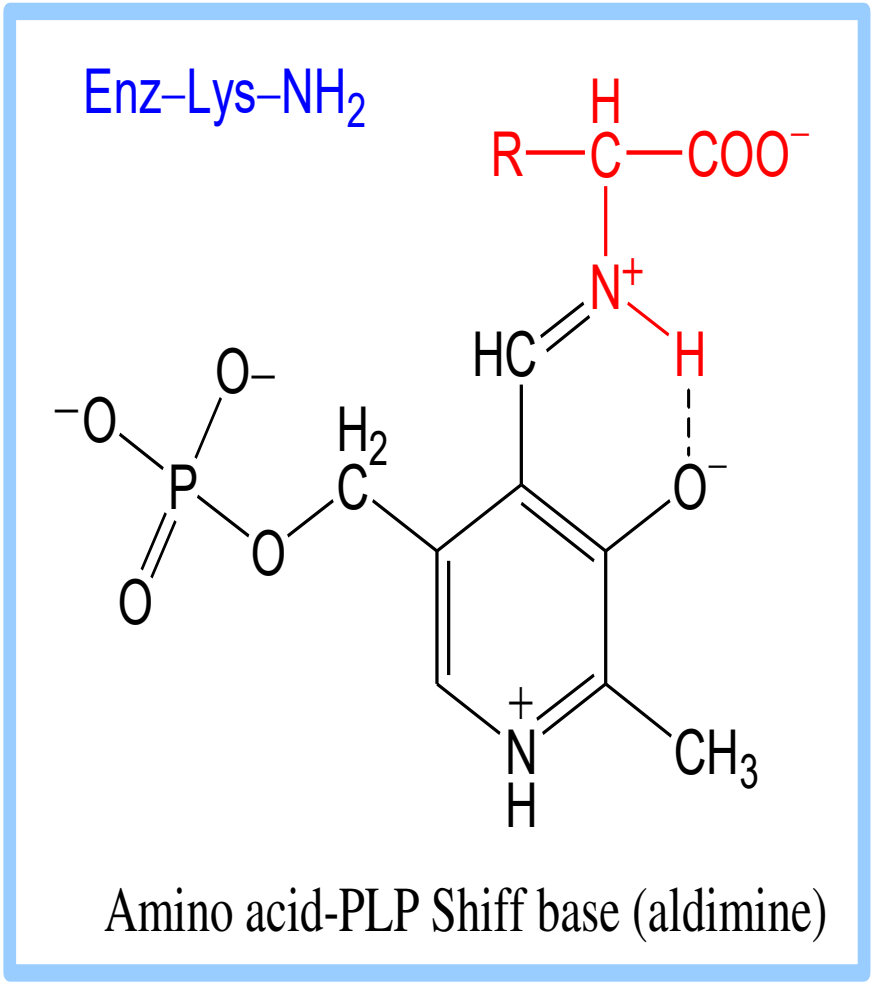


Enzyme (Lys)-PLP Schiff base

Pyridoxal Phosphate is bound to the enzyme through noncovalent interactions and a Schiff-base (aldimine) linkage to a Lys - residue at the active site.



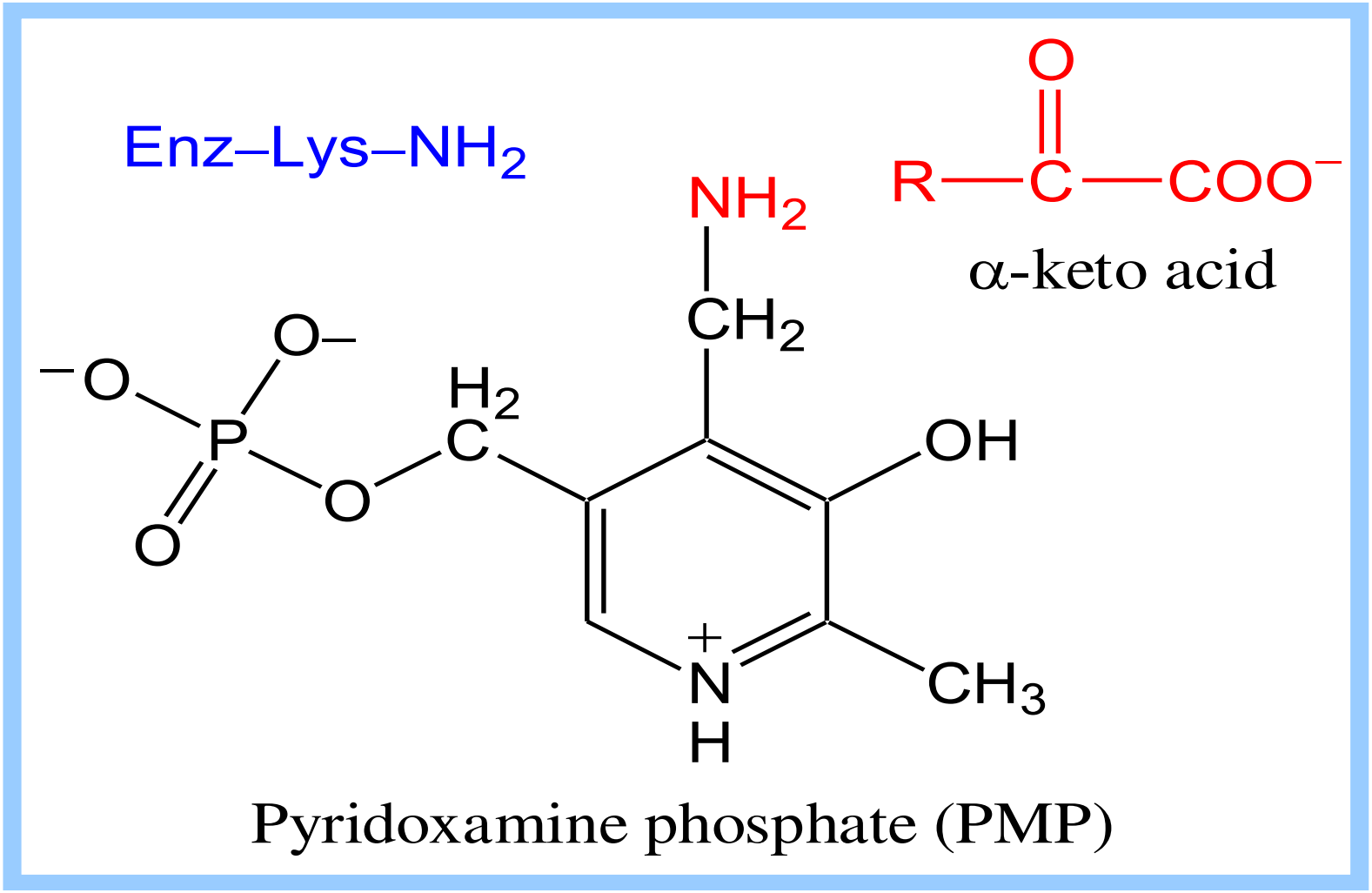
The α-amino group of a substrate **amino acid** displaces the enzyme lysine, to form a Schiff base I linked to PLP . The active site lysine extracts H^+, promoting tautomerization, followed by reprotonation & hydrolysis



Amino acid-PLP Shiff base (aldimine)

PLP to PMP

(Schiff base II reacts with water to dissociate into PMP & Keto acid)



Significance:

- Important method of Nitrogen catabolism of amino acids
- Synthesis of **new amino acids** from keto acids
- Produces **Pyruvic acid** and **Oxaloacetic acid** which are used in gluconeogenesis.

Deamination:

- The α- amino group of an amino acid is converted into ammonia while the amino acid itself converts into its corresponding keto acid.

Types:

1. Oxidative deamination
2. Non oxidative deamination
3. Transdeamination

Oxidative deamination

1. By L-amino acid oxidase:

L-amino acids are oxidatively deaminated in mitichondria, ER, peroxisome of kidneys.

It can not act on glycine and L- isomers of S-containing, hydroxy, dicarboxylic and basic amino acids.

It contains FMN as the prosthetic group.

Oxidative deamination

2. By D-amino acid oxidase:

Present in peroxisomes of mammalian liver and kidneys.

It can not act on D isomers of glutamic acid, asparagine, di-carboxylic and basic amino acids.

It contains FAD as prosthetic group. Its mode of action is comparable to L-amino acid oxidase

Oxidative deamination

3. By Glysine oxidase:

Glycine is oxidatively deaminated by hepatic glycine oxidase (West & Todd 1966).

It possess FAD as the prosthetic group.

Mechanism:

1. Dehydrogenation of amino acid. Amino acid oxidized to imino acid & FMN reduced to FMN.H2
2. Imino acid reacts spontaneously with water & dissociates into keto acid and ammonia.

Non oxidative deamination

- Molecular oxygen is not required for deamination

1. By amino acid dehydratase: Catalyze dehydration followed by deamination of hydroxy amino acids like serine & threonine.
2. By amino acid lyase: L –histidine & L- aspertic acid are deaminated byC-N amino acid lyase
3. By amino acid desulphydrase: Sulphur containing amino acid cysteine is deaminated by desulphydrase in presence of water
4. By transsulfurase: It catalyzespartial deamination of cystine in presence of water, to yeild pyruvic acid, ammonia & thiocysteine.

Trans-deamination

- A cyclical process in which-

1. Transamination takes place: in which mitochondrial & cytosolic transaminase of hepatocyte, transfer the α- amino group of a L - amino acid to α- ketogluteric acid. Glutamic acid is synthesized.
2. Oxidative deamination of Glutamic acid by mitochondrial GDH takes place, it utilizes NAD+ as hydrogen acceptor. Resulting in reproduction of α- ketogluteric acid which is recycled again.

THANK YOU
FOR
YOUR ATTENTION