

Protein Catabolism

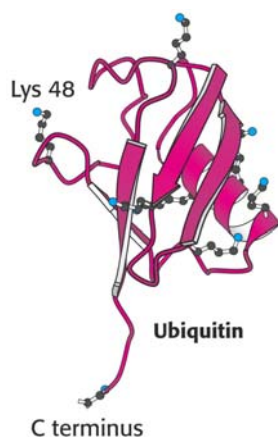
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The biosynthesis of proteins requires a continuous source of amino acids. Amino acids also provide cells with a source of nitrogen for the synthesis of nitrogen containing biomolecules such as nucleotides. Amino acids are generated by the digestion of proteins in the intestine or by the degradation of proteins within the cell. Cellular proteins are constantly being degraded and resynthesized. Some proteins are incredibly stable, others are very short lived. The short lived proteins usually play important metabolic roles. The short life times of these proteins allow the cell to rapidly adjust to changes in the metabolic state of the cell. An example of this is HMG-CoA reductase which catalyzes the first committed step of cholesterol biosynthesis. This enzyme has a half life of 3 hrs. Ornithine carboxylase has a half life of 11 minutes making it one of the shortest lived proteins.

I. Digestion of Dietary Proteins

Protein digestion begins in the stomach. The highly acidic environment of the stomach denatures proteins. Denatured proteins are susceptible to proteolytic digestion. The primary enzyme involved in proteolytic digestion is pepsin which catalyzes the nonspecific hydrolysis of peptide bonds at an optimal pH of 2. In the lumen of the small intestine, the pancreas secretes zymogens of trypsin, chymotrypsin, elastase ect. This battery of proteolytic enzymes breaks the proteins down into free amino acids as well as dipeptides and tripeptides. The free amino acids as well as the di and tripeptides are absorbed by the intestinal mucosa cells which subsequently are released into the blood stream where they are absorbed by other tissues.

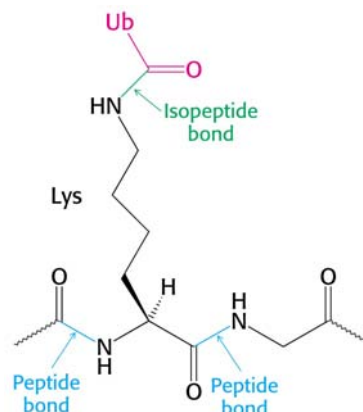


At the carboxyl terminus of ubiquitin, there is a glycine residue. This carboxylate group becomes attached to the ϵ -amino groups of several lysine residues on a protein destined for degradation.

The energy for the formation of this isopeptide bond comes from ATP.

II. Turnover of Cellular Proteins

Cellular proteins are continually being synthesized and degraded cell. How does the cell distinguish between functional proteins and old proteins that need to be degraded? Proteins are marked for degradation by the attachment of an Ubiquitin tag. Ubiquitin is a small protein found in all eukaryotic cells. Ubiquitin is attached to the terminal ϵ -amino group of lysine residues marking these proteins for degradation.

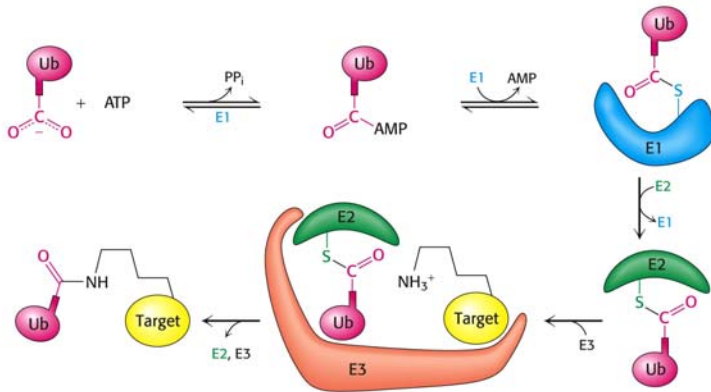


Three enzymes are involved in the tagging of a protein.

E1, The ubiquitin activating enzyme

E2, Ubiquitin conjugating enzyme

E3, Ubiquitin protein ligase.



First, the terminal carboxyl group of ubiquitin reacts with ATP to form a protein adenylylated intermediate and pyrophosphate. The adenylylated carboxyl group is then attacked by cysteine residue of E1 to form a thioester bond. This reaction is catalyzed by the ubiquitin activating enzyme. This reaction is analogous to the activation of fatty acids by acyl-CoA synthetase.

Second, the acyl group is transferred from the sulfhydryl of E1 to a cysteine residue of E2. This reaction is catalyzed by ubiquitin conjugating enzyme.

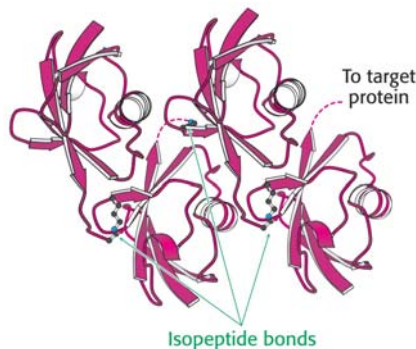
Third. Ubiquitin protein ligase catalyzes the transfer of ubiquitin from the sulfhydryl residue of E2 to the target protein.

The attachment of a single ubiquitin is a weak signal for degradation.

Ubiquitin has a lysine residue Lys-48 which is a site for the attachment of additional ubiquitin molecules.

Chains of 4 or more ubiquitins linked together are formed by the conjugation of the Lys-48 and the C-terminal carboxylate groups.

What determines whether or not a protein becomes ubiquinated?



This is a topic of on going research, but one simple correlation has become evident. The half life of a cytosolic protein is determined by a large extent by the N-terminal residue. The E3 enzymes interact with the amino-terminus which generates the selectivity.

Correlation of Half Life to the Amino Terminal Residue

Highly stabilizing residues

$t_{1/2} > 20$ hours

Ala, Cys, Gly, Ser, Met, Pro, Thr and Valine

Intrinsically destabilizing residues

$t_{1/2} = 2$ to 30 minutes

Arg, His, Ile, Leu, Lys, Phe, Trp and Tyr

Destabilizing Residues after Chemical Modification

$t_{1/2} = 3$ to 30 minutes

Asn, Asp, Gln, Glu

III. Proteasome

Once a protein is marked for degradation, the protein that executes the proteolysis is the proteasome. This humongous protein uses the energy of ATP to hydrolyze the peptide bonds of proteins. The proteasome has a sedimentation coefficient of 26S and is composed of 2 subunits, a 20S proteasome which contains all of the catalytic machinery to digest proteins and a 19S regulatory subunit. The 20S complex contains 2 copies of 14 subunits. The subunits are arranged into 4 rings or 7 subunits to form a stack that resembles a barrel. The two rings found at the ends of the barrel are called the α -subunits, the 2 central rings are called the β -subunits. The active sites are found in the N-terminal of the β -subunits. These active sites contain a threonine or serine residue that functions as nucleophile to attack the carbonyl bonds of proteins to form acyl-enzyme intermediates which are then hydrolyzed by water. The substrate proteins are degraded in a processive manner until the entire protein has been reduced too peptides of 7 to 9 residues.

The exact role of ATP hydrolysis is still under investigation. ATP hydrolysis may be involved in unfolding the protein or for the induction of conformational changes of the 20S complex. The 19S regulatory subunit contains the isopeptidase that removes the ubiquitin tag allowing the ubiquitin to label another protein for degradation.

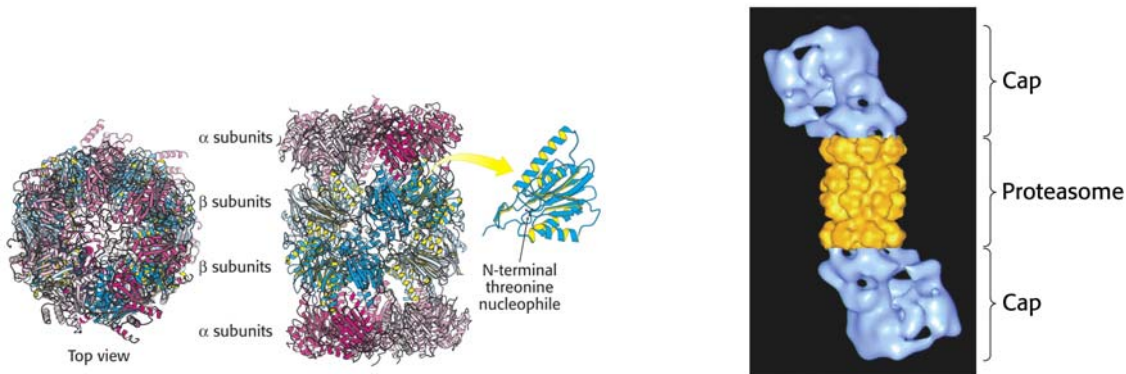


TABLE 23.2 Processes regulated by protein degradation

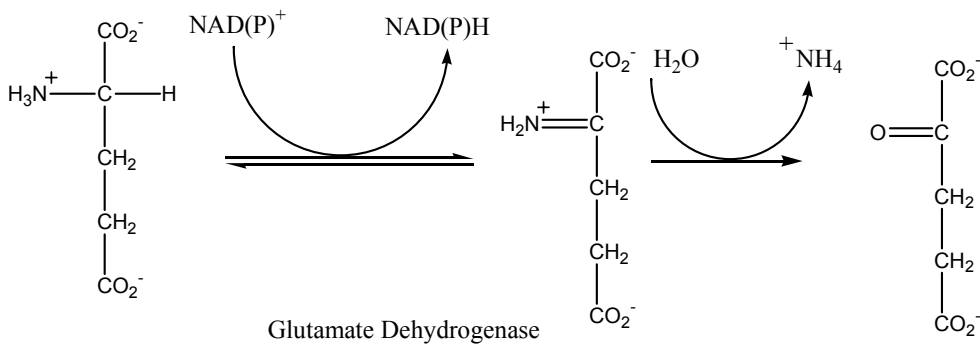
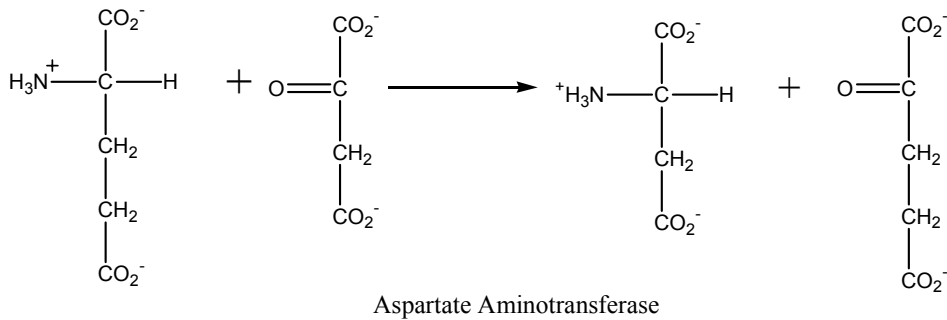
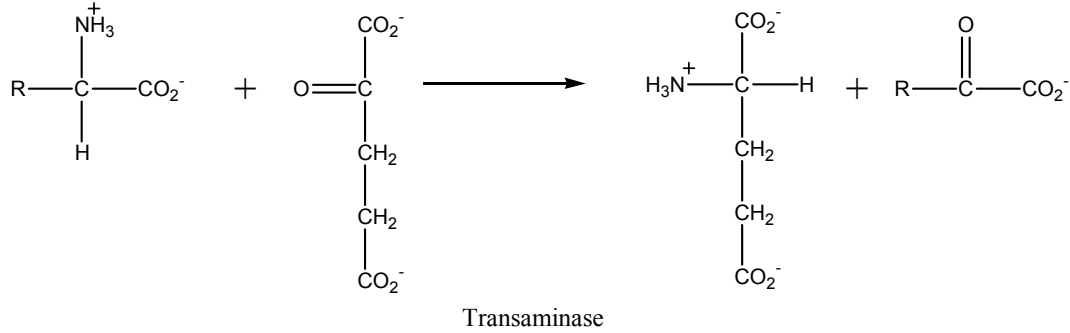
Gene transcription
Cell-cycle progression
Organ formation
Circadian rhythms
Inflammatory response
Tumor suppression
Cholesterol metabolism
Antigen processing

The peptide products are further degraded by cellular proteases to yield the individual amino acids. What is the fate of these amino acids? If amino acids are required for protein biosynthesis, the amino acids are utilized for that purpose. Otherwise they are degraded. In mammals, amino acid degradation occurs in the liver. The first step is to deaminate the amino acids to form α -ketoacids. The α -ketoacids are metabolized so that the carbon skeletons can enter the metabolic mainstream as precursors for gluconeogenesis or as citric acid cycle intermediates. Many physiological processes are regulated by protein digestion.

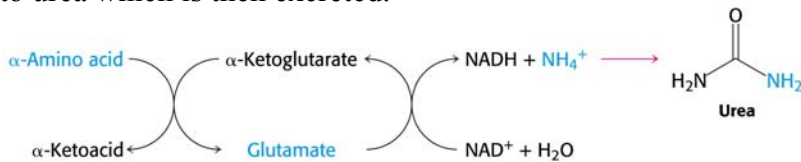
IV. Transaminations

The α -amino group of many amino acids is transferred to α -ketoglutarate to form glutamate. Glutamate can transfer the amino group to oxaloacetate to form aspartate or glutamine can undergo oxidative deamination to produce α -ketoglutarate and ammonia as shown on the next page. The enzymes that catalyze these reactions are called aminotransferases or transaminases. Aspartate aminotransferase

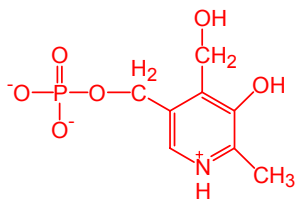
catalyzes the transfer of an amino group from aspartate to α -ketoglutarate to form oxaloacetate and aspartate. This transamination reaction is reversible. This enzyme will catalyze the transfer of an amino group from glutamate and transfer it to oxaloacetate to form aspartate and α -ketoglutarate. The nitrogen transferred to α -ketoglutarate in the transamination reaction can be converted into ammonia by oxidative deamination catalyzed by glutamate dehydrogenase which is an unusual enzyme in that it can use NAD^+ or NADP^+ as the oxidizing agent. The oxidation produces α -iminoglutarate which is a Schiff base that is hydrolyzed to form ammonia and α -ketoglutarate.



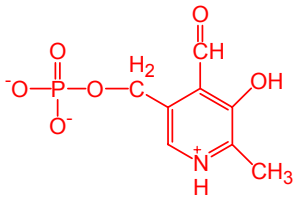
The equilibrium of the glutamate dehydrogenase reaction favors glutamate formation, but the reaction is driven forward as written by the consumption of ammonia. Most terrestrial vertebrates convert ammonia into urea which is then excreted.



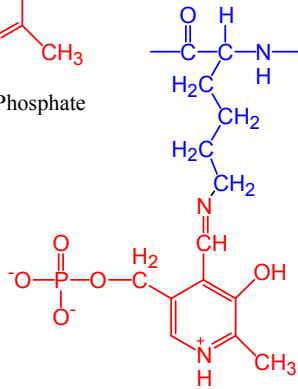
V. Pyridoxal Phosphate



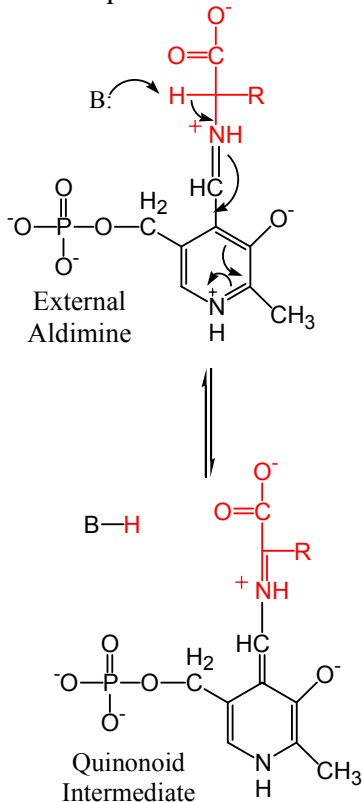
Pyridoxine
Vitamin B6



Pyridoxal Phosphate

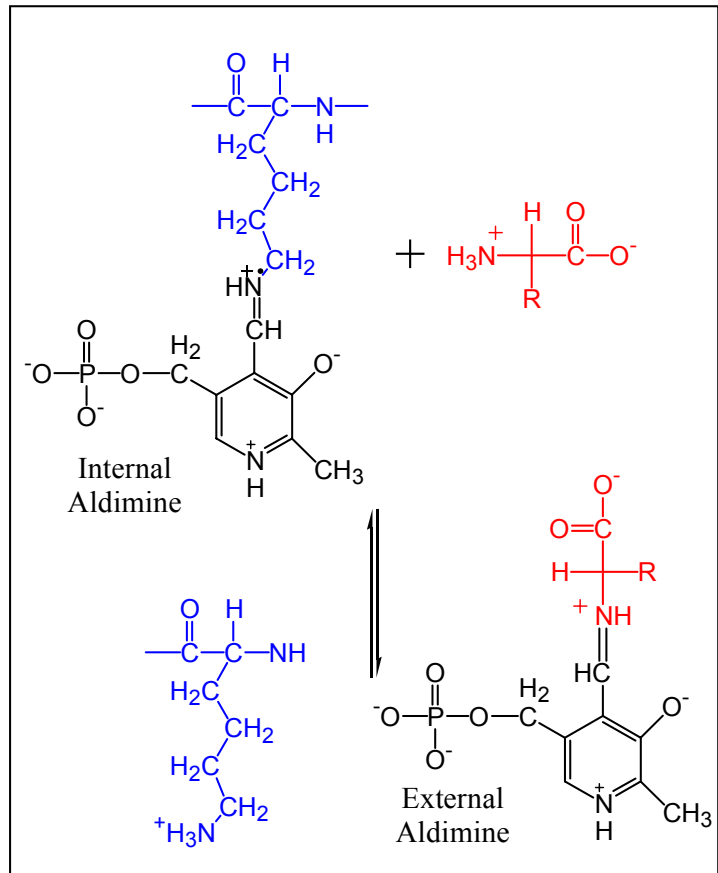


For the deamination reaction, the next step is abstraction of the proton on the α -carbon to form a quinonoid intermediate.

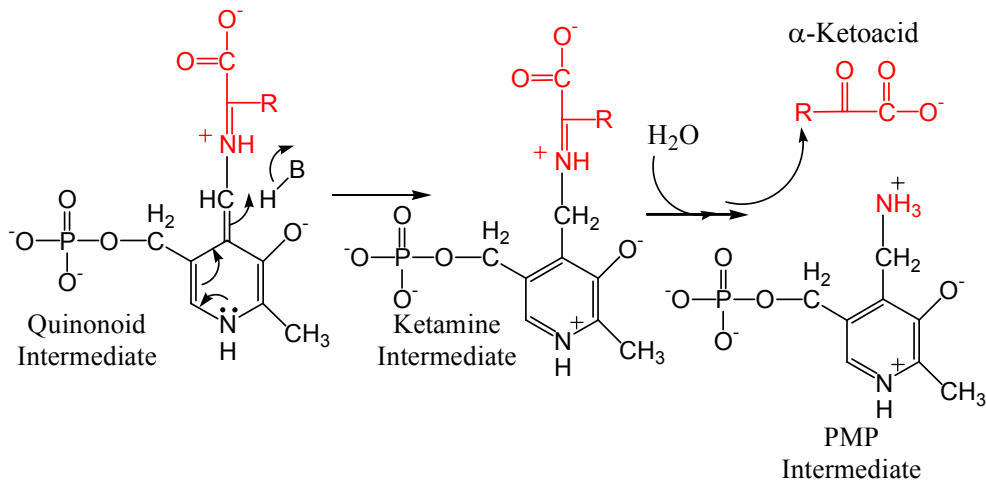


All of the aminotransferases contain a prosthetic group, pyridoxal phosphate (PLP). Pyridoxal phosphate is derived from vitamin B6, pyridoxine. Pyridoxal phosphate contains a pyridine ring that contains a phenolic hydroxyl group.

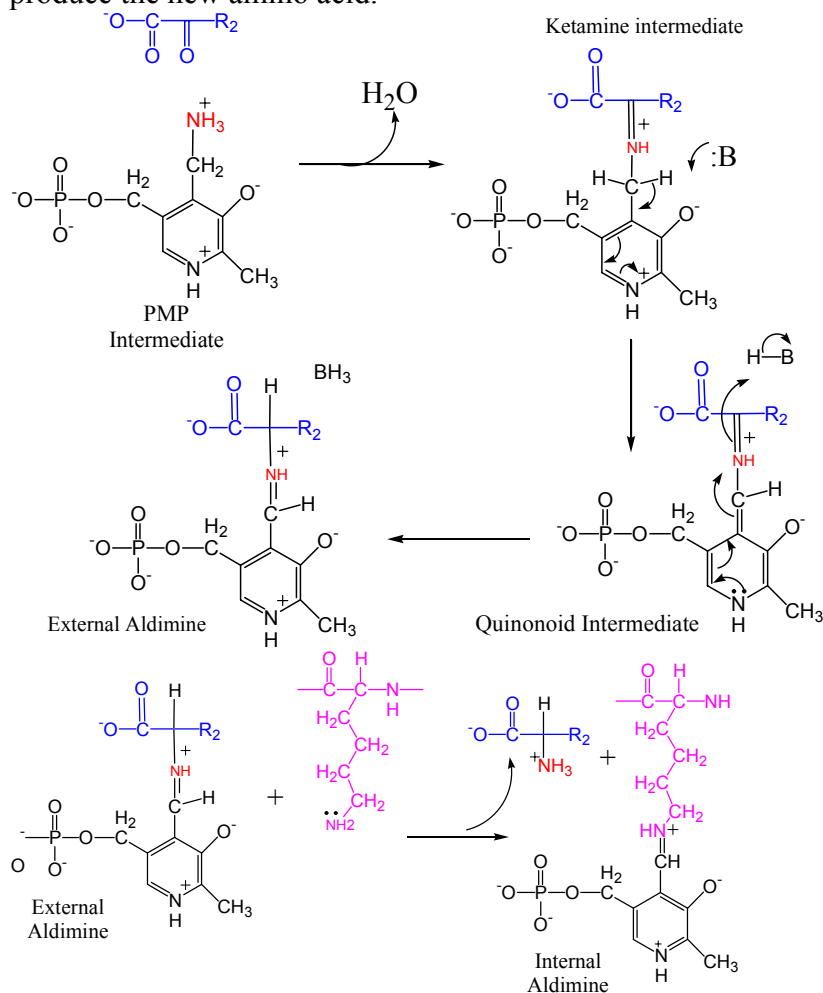
The aldehyde functionality of pyridoxal phosphate allows PLP to form a Schiff base linkage with the ϵ -amino group of a lysine residue of the enzyme. This Schiff base is called an internal aldimine. A new Schiff base linkage is formed upon the addition of an amino acid substrate. This Schiff base linkage is called an external aldimine. The α -amino group of the amino acid substrate is a nucleophile that attacks the carbon of the Schiff base and displaces the lysine residue.



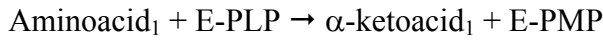
The negative charge generated by the proton extraction is stabilized by the electron sink provided by the pyridinium ring. The quinonoid intermediate is reprotonated at the aldehyde carbon atom to yield a ketimine intermediate. The ketimine intermediate is then hydrolyzed to produce an α -ketoacid and a pyridoxamine phosphate (PMP).



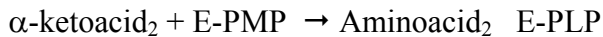
This completes the first half of the transamination reaction. The amino acid has been converted to an α -ketoacid. The amino group is attached to the pyridoxal phosphate as PMP. The second half of the reaction is the exact reverse of the first half. A second α -ketoacid reacts with PMP to form a Schiff base generating a ketamine intermediate. A general base abstracts the proton from the aldehyde carbon to regenerate the quinonoid intermediate. A proton is then donated to the α -carbon to generate the external aldimine. The active site lysine then regenerates the Schiff base linkage to form the internal aldimine and produce the new amino acid.



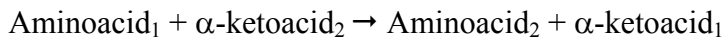
The Reaction for the first half reaction:



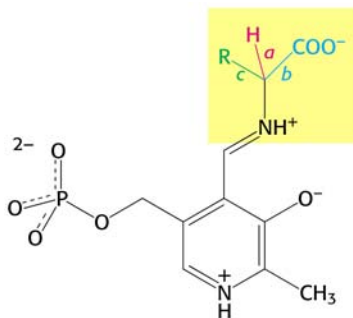
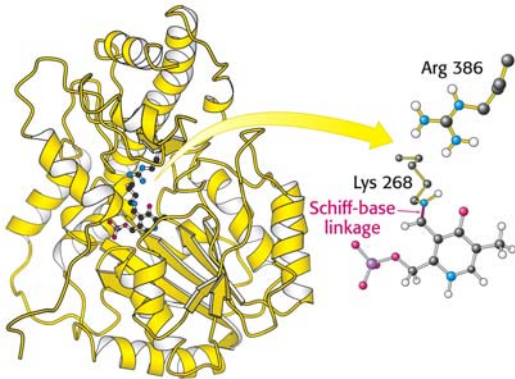
The Second half Reaction:



The net reaction:



Aspartate Aminotransferase is a typical pyridoxal dependent enzyme. The crystal structure of this enzyme has been solved. The lysine that forms the Schiff base with the aldehyde of pyridoxal phosphate is Lys-268. Adjacent to the pyridoxal cofactor is the binding site for aspartate/oxaloacetate. When aspartate binds to the active site of the enzyme the α -amino group displaces Lys-268 to form the external aldimine. The next step in the enzyme catalyzed pathway is the abstraction of the proton from the α -carbon to generate the quinonoid intermediate. The general base that abstracts this proton is the same Lys-268. The protonated Lys-268 then transfers this proton to the aldehyde carbon to generate the ketamine intermediate.



Other Reactions Catalyzed by PLP Enzymes

Transamination is just one of the reactions catalyzed by PLP enzymes. Once the external aldimine has been formed with an amino acid substrate, there are a variety of transformations that can occur.

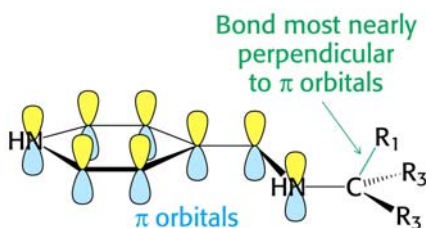
- Transaminations
- Decarboxylations
- Racemizations
- Aldol Cleavages
- Eliminations and Replacement Reactions

All of these PLP dependent reactions share the following similarities.

1. A Schiff base is formed by the amino acid substrate and PLP.
2. PLP acts as an electron sink to stabilize the development of negative charge in the transition state. PLP is an electrophilic catalyst.
3. The product is in the form of a Schiff base with PLP which is cleaved at the completion of the reaction.

PLP enzymes cleave one of the 3 bonds labeled a, b, c. In the transaminase reactions the a-bond to the hydrogen is cleaved. In the decarboxylation reactions, the b-bond. PLP dependent aldolases cleave bond c.

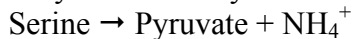
Different enzymes favor the cleavage of one of the three bonds by orienting it perpendicular to the pyridinium ring. So for the aminotransferases, the C_{α} -H bond is held perpendicular to the PLP ring. In this way PLP enzymes control the possible catalytic outcomes.



VI. Deamination of Serine and Threonine.

Most deaminations of amino acids transfer the amino group to an α -ketoacid. Serine and threonine on the other hand can be directly deaminated by serine dehydratase or threonine dehydratase.

Serine dehydratase and **threonine dehydratase** both contain a PLP prosthetic group. Serine dehydratase catalyzes the following reaction:



Threonine dehydratase catalyzes a similar reaction:



Both enzymes use the same mechanism. The PLP dependent reaction forms an **aminoacrylate** intermediate which spontaneously deaminates. The mechanism shown below is for Serine Dehydratase:

