Amino Acids Metabolism Part II

Conversion of amino acids to specialized products

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Nitrogen metabolism

Atmospheric nitrogen N2 is most abundant but is too inert for use in most biochemical processes.

Atmospheric nitrogen is acted upon by bacteria (nitrogen fixation) and plants to nitrogen containing compounds. We assimilate these compounds as proteins (amino acids) in our diets.

Conversion of nitrogen into specialized products

Conversion of nitrogen into specialized products

Disposal of Nitrogen

Lecture I

Lecture II

Lecture III

Amino acids synthesis & degradation

Amino acids synthesis & degradation

Enters various metabolic pathways

Enters various metabolic pathways

Excreted

Excreted

Body proteins

Body proteins

Other nitrogen containing compounds

Other nitrogen containing compounds

α-amino groups

α-amino groups

Carbon skeletons

Carbon skeletons

NH4+

NH4+

Urea

Urea

α-amino groups

α-amino groups

Amino acids

Amino acids

Dietary proteins

Dietary proteins

N2

N2

Metabolism Summary

Metabolism Summary

- Proteins (amino acids)
- Carbohydrates (glucose, fructose, galactose)
- Fats and lipids (fatty acids, triglycerides)
- Urea cycle (NH3, CO2, urea)
- Electron transport chain (NADH, FADH2, ATP)
- Cyclic Acid Cycle (NADH, FADH2, ATP)
- Pyruvic Acid (NADH, FADH2, ATP)
- Lipogenesis (NADH, FADH2, ATP)
Amino Acids as precursors of nitrogen-containing compounds
Porphyrin metabolism
Porphyrrins are cyclic compounds that bind metal ions, usually Fe$^{2+}$ or Fe$^{3+}$

The most common metalloporphyrin is heme

A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin

<table>
<thead>
<tr>
<th>Protein</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>Transport of oxygen in blood</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>Storage of oxygen in muscle</td>
</tr>
<tr>
<td>Cytochrome c</td>
<td>Involvement in electron transport chain</td>
</tr>
<tr>
<td>Cytochrome P450</td>
<td>Hydroxylation of xenobiotics</td>
</tr>
<tr>
<td>Catalase</td>
<td>Degradation of hydrogen peroxide</td>
</tr>
<tr>
<td>Tryptophan pyrrolase</td>
<td>Oxidation of tryptophan</td>
</tr>
</tbody>
</table>

Example of some human and animal heme proteins
1) Porphyrins contain four pyrrole rings joined through methylene bridges.

2) Side chains differs in different porphyrine.
   - Uroporphyrins contains acetate(-CH$_2$-COO$^-$) and propionate (-CH$_2$-CH$_2$-COO$^-$) side chains
   - Coproporphyrins contains methyl and propionate groups.
   - Protoporphyrins IX (and heme) contains vinyl, methyl and propionate groups.

3) Side chains are ordered around porphyrine tetrapyrrole nucleus in four different ways designated as roman letters I-IV.

4) These side chains are either symmetrically or asymmetrically ordered on pyrrole rings.
   - e.g. Type I uroporphyrins I, A acetate alternates with P (propionate) around the tetrapyrrole ring.

5) Type III porphyrines (e.g. uroporphyrin III) which contain an asymmetric substitution on ring D are physiologically important in humans.

Porphyrinogens: porphyrin precursors, intermediate between porphobilinogen and the oxidized colored protoporphyrins in heme biosynthesis.
Heme synthesis occurs in all cells due to the requirement for heme as a prosthetic group on enzymes and electron transport chain proteins. By weight, the major locations of heme synthesis are the liver (cytochrome p450) and the erythroid progenitor cells (Hemoglobin) of the bone marrow.
Overview of Heme Synthesis

Succinyl CoA + Glycine → δ-aminolevulinic acid

ALA synthase → δ-aminolevulinic acid

δ-aminolevulinic acid → Porphobilinogen → Uroporphyrinogen III → Coproporphyrinogen III → Protoporphyrinogen IX → Protoporphyrin IX → Heme

Cytoplasm mitochondrial matrix

Mature red blood cells lack mitochondria and are unable to synthesize heme
1) Formation of δ-aminolevulinic acid (ALA) (In mitochondria)

All the carbon and nitrogen atoms of porphyrin molecules are provided by Glycine (non essential aa) and Succinyl CoA (an intermediate in the citric acid cycle).

Glycine and succinyl CoA condense to form ALA, a reaction catalyzed by ALA synthatase. This reaction requires pyridoxal phosphate as a coenzyme.

When porphyrin production exceeds the availability of globin, heme accumulates and is converted to hemin by oxidation of Fe2+ to Fe3+.

Hemin negatively regulates ALA by decreasing synthesis of hepatic ALA synthase enzyme

Many drugs (e.g. antifungal, anticonvulsants) increase ALA synthesis. Because these drugs are metabolized in liver by Cyt. P450, a heme containing enzyme. This results in increase synthesis of Cyt. P450, leading to consumption of heme.

In erythroid cells heme synthesis is under the control of erythropoietin and the availability of iron

2) Formation of porphobilinogen (In cytosol)

Two molecules of ALA condenses to form porphobilinogens by ALA dehydratase, the reaction sensitive to heavy metal ions.
Biosynthesis of heme

3) Formation of uroporphyrinogen (In cytosol)

The condensation of four molecules of porphobilinogens results in the formation of tetrapyrrole, hydroxymethylbilane, a reaction catalyzed by hydroxymethylbilane synthase.

Isomerization and cyclization by uroporphyrinogen III synthase leads to the formation of Uroporphyrinogen III.

Uroporphyrinogen III undergoes decarboxylation at its acetate groups, generating coproporphyrinogen III, a reaction carried out by uroporphyrinogen decarboxylase.

Two propionate side chains are decarboxylated to vinyl groups generating protoporphyrinogen IX, which is then oxidized to protoporphyrin IX.
Biosynthesis of heme

4) Formation of heme (In mitochondria)
Introduction of iron (as Fe2+) occurs spontaneously but the rate is enhanced by ferrochelatase. This enzyme like ALA is also inhibited by lead.

![Diagram of heme biosynthesis](image)
Overview of Heme Synthesis

Succinyl CoA + Glycine → \(\delta\)-aminolevulinic acid

\(\delta\)-aminolevulinic acid → Porphobilinogen → Uroporphyrinogen III → Coproporphyrinogen III

Protoporphyrinogen IX → Protoporphyrin IX → Coproporphyrinogen III

Heme

Mature red blood cells lack mitochondria and are unable to synthesize heme
Porphyrias

Purple color caused by pigment-like porphyrins in the urine

Porphyrias is caused due to the inherited (or occasionally acquired) defects in heme synthesis.

- Leads to the accumulation and increased excretion of porphyrins and porphyrins precursors.
- Mutations that cause porphyria are heterogenous (not all the same DNA locus).
- Each porphyria leads to accumulation of a unique pattern of intermediates.
- Porphyrias are classified as erythropoietic (enzyme deficiency is in the erythropoietic cell) or hepatic (enzyme deficiency is in the liver).

Hepatic

Chronic  Acute

Porphyrin accumulation leads to cutaneous symptoms and urine that is red to brown in natural light and pink to red in fluorescent light.

Neurological, cardiovascular, symptoms
Abdominal pain
<table>
<thead>
<tr>
<th>Name</th>
<th>Deficient enzyme</th>
<th>Accumulated Intermediates</th>
<th>Photosensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute intermittent porphria <em>(Acute)</em></td>
<td>Hydroxymethylbilane synthtase</td>
<td>Protoporphyrin and ALA in the urine</td>
<td>-</td>
</tr>
<tr>
<td>Variegate porphyria <em>(Acute)</em></td>
<td>Protoporphyrinogen oxidase</td>
<td>Protoporphyrinogen IX and other intermediates prior to the block in the urine</td>
<td>+</td>
</tr>
<tr>
<td>Heriditary Coproporphyria <em>(Acute)</em></td>
<td>Coproporphyrinogen oxidase</td>
<td>Coproporphyrinogen III other intermediates prior to the block in the urine</td>
<td>+</td>
</tr>
<tr>
<td>Erythropoietic porphyria</td>
<td>Ferrochelatase</td>
<td>Protoporphyrins accumulate in the Bone marrow, erythrocytes and plasma</td>
<td>+</td>
</tr>
<tr>
<td>Erythropoietic protoporphryia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Congenital Erythropoietic porphryia</td>
<td>Uroporphyrinogen III synthtase</td>
<td>Uroporphyrinogen I and coporphyrinogen I urine</td>
<td>+</td>
</tr>
<tr>
<td>Hepatic and Erythropoietic porphyria</td>
<td>Uroporphyrinogen decarboxylase</td>
<td>Uroporphyrinogen I and coporphyrinogen I in urine</td>
<td>+</td>
</tr>
<tr>
<td>Enzyme Involved2</td>
<td>Type, Class, and MIM Number</td>
<td>Major Signs and Symptoms</td>
<td>Results of Laboratory Tests</td>
</tr>
<tr>
<td>------------------</td>
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<td>----------------------------</td>
</tr>
<tr>
<td>1. ALA synthase</td>
<td>X-linked sideroblastic anemia3 (erythropoietic) (MIM 301300)</td>
<td>Anemia</td>
<td>Red cell counts and hemoglobin decreased</td>
</tr>
<tr>
<td>2. ALA dehydratase</td>
<td>ALA dehydratase deficiency (hepatic) (MIM 125270)</td>
<td>Abdominal pain, neuropsychiatric symptoms</td>
<td>Urinary ALA and coproporphyrin III increased</td>
</tr>
<tr>
<td>3. Uroporphyrinogen I synthase4</td>
<td>Acute intermittent porphyria (hepatic) (MIM 176000)</td>
<td>Abdominal pain, neuropsychiatric symptoms</td>
<td>Urinary ALA and PBG increased</td>
</tr>
<tr>
<td>4. Uroporphyrinogen III synthase</td>
<td>Congenital erythropoietic (erythropoietic) (MIM 263700)</td>
<td>No photosensitivity</td>
<td>Urinary, fecal, and red cell uroporphyrin I increased</td>
</tr>
<tr>
<td>5. Uroporphyrinogen decarboxylase</td>
<td>Porphyria cutanea tarda (hepatic) (MIM 176100)</td>
<td>Photosensitivity</td>
<td>Urinary uroporphyrin I increased</td>
</tr>
<tr>
<td>6. Coproporphyrinogen oxidase</td>
<td>Hereditary coproporphyria (hepatic) (MIM 121300)</td>
<td>Photosensitivity, abdominal pain, neuropsychiatric symptoms</td>
<td>Urinary ALA, PBG, and coproporphyrin III and fecal coproporphyrin III increased</td>
</tr>
<tr>
<td>7. Protoporphyrinogen oxidase</td>
<td>Variegate porphyria (hepatic) (MIM 176200)</td>
<td>Photosensitivity, abdominal pain, neuropsychiatric symptoms</td>
<td>Urinary ALA, PBG, and coproporphyrin III and fecal protoporphyrin IX increased</td>
</tr>
<tr>
<td>8. Ferrochelatase</td>
<td>Protoporphyrria (erythropoietic) (MIM 177000)</td>
<td>Photosensitivity</td>
<td>Fecal and red cell protoporphyrin IX increased</td>
</tr>
</tbody>
</table>
Porphyrias Contd-----

**Lead poisoning**

*Ferrochelatase and ALA synthase are inhibited*
*Protoporphyrin and ALA accumulate in urine*

**Photosensitivity**

It is due to the porphyrin-mediated formation of superoxide radicals from oxygen. These reactive species can oxidatively damage membranes, and cause the release of lysosomal enzymes. Destruction of cellular components cause photosensitivity.
One common feature of porphyria is decrease synthesis of heme causing increase in ALA synthetase activity.

Treatment: Intravenous injection of hemin to decrease the synthesis of ALA synthetase.
Degradation of heme

RBCs last for 120 days and are degraded by reticuloendothelial (RE) system [liver and spleen]. About 85% of heme destined for degradation comes from RBCs and 15% from cytochromes, and immature RBCs.

1) Formation of bilirubin
   a) Microsomal heme oxygenase hydroxylates methenyl bridge between two pyrrole rings with concomitant oxidation of Fe$^{2+}$ to Fe$^{3+}$.
   b) A second oxidation by the same enzyme results in the cleavage of the porphyrin ring resulting in biliverdin (green color).
   c) Biliverdin is then reduced by biliverdin reductase, forming the bilirubin (red-orange).

2) Uptake of bilirubin by liver
   Bilirubin then binds to serum albumin and is transported to the liver.

3) Formation of bilirubin diglucuronide
   Bilirubin is then conjugated to two molecules of glucuronic acid by the enzyme bilirubin glucuronyl-transferase using UDP-glucuronic acid as a glucuronate donor (to increase the solubility of bilirubin)

4) Secretion of bilirubin into bile
   Conjugated form of bilirubin is the secreted into the bile.

5) Formation of urobilins
   Bilirubin diglucuronide is hydrolyzed and reduced by bacteria in the gut to yield Urobilinogen-------oxidized to stercobilin.
Catabolism of heme

1. Senescent red cells are a major source of hemeproteins.

2. Breakdown of heme to bilirubin occurs in macrophages of the reticulo-endothelial system (tissue macrophages, spleen, and liver).

3. Unconjugated bilirubin is transported through the blood (complexed to albumin) to the liver.

4. Bilirubin is taken up by the liver and conjugated with glucuronic acid.

5. Bile is secreted from the liver into the intestine.

6. In the intestine, glucuronic acid is removed by bacteria. The resulting bilirubin is converted to urobilinogen.

7. Some of the urobilinogen is reabsorbed from the gut and enters the portal blood.

8. A portion of this urobilinogen participates in the enterohepatic urobilinogen cycle.

9. The remainder of the urobilinogen is transported by the blood to the kidney, where it is converted to yellow urobilin and excreted, giving urine its characteristic color.

10. Urobilinogen is oxidized by intestinal bacteria to the brown stercobilin.
Yellow color of the skin, nailbeds, and sclerae (whites of the eyes) caused due to deposition of Bilirubin.
Liver can handle 3000 mg bilirubin/day – normal production is 300 mg/day in liver.

Massive hemolysis leads to increase degradation of heme, and therefore production of bilirubin. Bilirubin therefore cannot be conjugated.

Increased bilirubin is excreted into bile, urobilinogen is increased in blood, urine. Unconjugated bilirubin in blood increases = jaundice.

Obstruction of the bile duct (due to the hepatic tumor, or bile stones) prevents passage of bilirubin into intestine. Prolonged obstruction of the bile duct can lead to liver damage and a subsequent increase in unconjugated Bilirubin.

Damage to liver cells leads to decrease in glucuronidin Conjugation and increase in unconjugated bilirubin.

Premature babies often accumulate bilirubin due to late onset of expression of hepatic bilirubin Glucuronyltransferase (BG). This enzyme is normally low at birth and reaches adult levels in about four weeks.

Newborns are treated with blue Fluorescent light, which converts bilirubin to water soluble isomers. These photoisomers can be excreted into the bile without conjugation to glucuronic acid.
Determination of Bilirubin concentration

Van der Bargh reaction

Diazotized sulfanilic acid + Bilirubin $\rightarrow$ Diazopyrroles (red color)

Measured Calorimetrically
Other nitrogen containing compounds

Catecholamines

Dopamine, norepinephrine (noradrenaline) and epinephrin (adrenaline) are biologically active amines and are collectively called as Catecholeamines.

* Dopamine and norepinephrine functions as a neurotransmitters.

Outside the nervous system, norepinephrine and its methylated derivative, epinephrine regulates carbohydrate and lipid metabolism.

They are released from storage vehicles in the adrenal medulla in response to stress (fright, exercise, cold, and low levels of blood glucose).

They increase the degradation of glycogen, and triglycerides, as well as increase blood pressure and the output of heart.
Synthesis of catecholamine

Catecholamines are synthesized from Tyrosine

- Tyrosine is hydroxylated by tyrosine hydroxylase (rate limiting step in the pathway) to form DOPA.
- DOPA is decarboxylated by DOPA decarboxylase (pyridoxal phosphate requiring enzyme) to form dopamine.
- Dopamine is then hydroxylated by Dopamine β-hydroxylase to give norepinephrine.
- Epinephrine is formed by N-methylation reaction using S-adenosylmethionine as a methyl donor.

Parkinson’s disease is caused due to the production of insufficient dopamine synthesis in brain
Degradation of catecholamines

The catecholamines are inactivated by oxidative deamination by **monoamine Oxidase (MAO)** and by O-methylation carried out by **catechol-O-methyl-transferase (COMT)** as the one-carbon donor.
- Two reactions can occur in either direction
- The aldehyde products of the MAO reaction are oxidized to the corresponding acids

MAO: inactivates catecholamines by oxidative deamination to yield the corresponding aldehyde

COMT: inactivates catecholamines by methylation using S-adenosylmethionine (SAM)

MAO inhibitors:
- Found in neural tissue, gut and liver
- Antidepressant
- Act by inhibiting MAOs
- Resulting in increase availability of neurotransmitters allowing their accumulation in the presynaptic neuron and subsequent leakage into circulation, providing an antidepressant action.

- Metabolic products of the reaction are excreted in the urine.
Histamines

-A chemical messenger that mediates a wide range of cellular responses, including allergic and inflammatory reactions, gastric acid secretion, and possibly neurotransmission in the brain.

They are secreted by mast cells as a result of allergic reactions or trauma.

Antihistamines are used to block histamine production during allergic reactions.
Serotonin (5-hydroxytryptamine)

- mostly found in the cells of intestinal mucosa
- smaller amounts occurs in CNS where it functions as a neurotransmitter
- also found in platelet
- has roles in pain perception, affective disorders, regulation of sleep, temperature, and blood pressure.

Also degraded by MAO.
Creatine (phosphocreatine)
- Found in muscle
- High energy compound that can donate phosphate group to ADP to form ATP
- Creatine is reversibly phosphorylated to creatine phosphate by creatine kinase.
- Creatine phosphate serves as a reserve of high-energy phosphates that can be used to maintain ATP levels.
- Levels of creatine kinase in plasma is an indicator of tissue damage and is used in the diagnosis of myocardial infarction.

**Synthesis**
- Synthesized from glycine, and the guanidino group of arginine, plus a methyl group from S-adenosylmethionine.
- Reversibly phosphorylated to creatine phosphate by creatine kinase using ATP as a phosphate donor.

**Degradation**
Both creatine and creatine phosphate cyclize to form creatinine which is then excreted in the urine.
**Melanin**

- Pigment that occurs in several tissues, e.g. in eye, skin, and hair.
- Synthesized from tyrosine in the epidermis by melaocytes
- Function is to protect tissues from sun-light
- Defect in melanin formation occurs in albinism due to the defective copper-containing enzyme tyrosinase.
I Amino Acids pool

Supplied

a) Degradation
   (Lysosomal and proteasome)
b) Dietary protein
c) Do novo synthesis

Depleted

a) Synthesis of body proteins
b) Precursors for essential N-containing molecules

II Digestion of dietary proteins
a) Gastric enzymes
b) Pancreatic enzymes
c) Small Intestinal enzymes (proteases cascade)
d) Amino acid specificity for proteolytic enzymes

III) How amino acids are transported into cells
Transport systems

IV) Removal of nitrogen from amino acids
a) Transamination (aminotransferases)
b) Oxidative deamination (Glutamine dehydrogenase)

V) Urea cycle
Reactions of urea cycle: a) locations b) sequence b) enzymes for each reaction c) end products for each reaction d) ATP requirements e) sources of nitrogens in urea

VI) Metabolism of ammonia
a) Sources of ammonia,
b) Transport of ammonia,
c) Urea cycle defects in humans
I) Essential and non essential amino acids
   Names of the essential and non essential aa

II) Glucogenic and ketogenic amino acids
   a) Why amino acids are classified as glucogenic and ketogenic or both?
   b) Seven intermediates of carbon skeleton
   c) Amino acids that form those intermediates

III) Catabolism of the branched-chain amino acids

IV) Biosynthesis of nonessential amino acids
   a) Synthesis from $\alpha$-keto acids
   b) Synthesis by amidation
   c) Synthesis of proline, serine, glycine, cysteine, tyrosine

V) Metabolic defects in amino acid metabolism
   a) Phenylketourea
   b) Maple Syrup urine disease
   c) Albinism
   d) Homocystinuria
   e) Alkaptouria

   Defective enzyme
   Amino acid involved
   Accumulated intermediate
   Characteristics
I) Amino acids as a precursors for:

Porphyrrines
Heme:
  a) Synthesis
  b) Degradation
  c) Diseases caused due to the defective heme synthesis & degradation (Jaundice)

Catecholamines (Dopamine, epinephrine, Norepinephrine)
  a) Synthesis
  b) Degradation

Histamine

Serotinine

Creatine

Melanine