Protein & Amino Acid Metabolism AND The Urea Cycle

Dr Piyush B. Tailor
Department of Biochemistry
Govt. Medical College
Surat
Three sources of amino acids

• Synthesis of Non-essential amino acid from metabolic intermediate.
• Breakdown of proteins.
• Amino acids derived from dietary protein.
Amino acid is depleted by three routes

• Synthesis of body protein
• Amino acids consumed as precursors of essential nitrogen-containing small molecules
• Conversion of amino acids to glucose, glycogen, fatty acids or CO2
Amino Acid Degradation must account for an amino group.
Protein Degradation

1. Ubiquitin - Proteasome
   Proteolytic enzyme

2. Chemical Signal for Protein Degradation
Ubiquitin – Proteasome Proteolytic enzyme

- first covalently attached to ubiquitin, a small globular protein.
- Through linkage of the Glycine of ubiquitin to a Lysine on protein substrate
- Proteins tagged with ubiquitin are targeted by proteasome, which functions like a garbage disposal.
- The proteasome cuts the target protein into fragments that are then further degraded to amino acids, which enter the amino acid pool.
- The ubiquitins are recycled.
Chemical Signal for Protein Degradation

- Because proteins have different half-lives, it is clear that protein degradation cannot be random.
- But rather is influenced by some structural aspect of the protein.
- For example, some proteins that have been chemically altered by oxidation or tagged with ubiquitin are preferentially degraded.
- The half-life of a protein is influenced by the nature of the N-terminal residue.
1. Protein selected for degradation is tagged with molecules of ubiquitin.

2. Ubiquinated proteins are recognized by the cytosolic proteasome, which unfolds and transports the protein to its proteolytic core.

3. Peptide fragments produced by the proteasome are degraded to amino acids.
The different forms of excreted nitrogen

- **Ammonia (as ammonium ion):** \( \text{NH}_4^+ \)
  - Ammonotelic animals: most aquatic vertebrates, such as bony fishes and the larvae of amphibia

- **Urea:** \( \text{H}_2\text{N}—\text{C}—\text{NH}_2 \)
  - Ureotelic animals: many terrestrial vertebrates; also sharks

- **Uric acid:**
  - Uricotelic animals: birds, reptiles
Protein Digestion
Monogastric Protein Digestion

- Whole proteins are not absorbed
  - Too large to pass through cell membranes intact

- Digestive enzymes
  - Break peptide bonds
- Secreted as inactive pre-enzymes
  - Prevents self-digestion
Dietary protein

Pepsin

Polypeptides and amino acids

Trypsin
Chymotrypsin
Elastase
Carboxypeptidase

Oligopeptides and amino acids

Aminopeptidases
Di- and tripeptidases

Amino acids
Monogastric Protein Digestion

• Initiated in stomach
  - HCl from parietal cells
    • Stomach pH 1.6 to 3.2
    • Denatures 4\(^0\), 3\(^0\), and 2\(^0\) structures
  - Pepsinogen from chief cells

\[
\text{Pepsinogen} \xrightarrow{\text{HCl}} \text{Pepsin}
\]

• Cleaves at phenylalanine, tyrosine, tryptophan

• Protein leaves stomach as mix of insoluble protein, soluble protein, peptides and amino acids
Protein Digestion – Small Intestine

- Pancreatic enzymes secreted
  - Trypsinogen
  - Chymotrypsinogen
  - Procarboxypeptidase
  - Proelastase
  - Collagenase

\[ \text{Zymogens} \]
Digestion - Small Intestine

- Zymogens must be converted to active form
  - Trypsinogen \( \xrightarrow{\text{Enteropeptidase/Trypsin}} \) Trypsin
    - Endopeptidase
      - Cleaves on carbonyl side of Lys & Arg
  - Chymotrypsinogen \( \xrightarrow{\text{Trypsin}} \) Chymotrypsin
    - Endopeptidase
      - Cleaves carboxy terminal Phe, Tyr and Trp
  - Procarboxypeptidase \( \xrightarrow{\text{Trypsin}} \) Carboxypeptidase
    - Exopeptidase
      - Removes carboxy terminal residues
Protein Digestion

- Small intestine (brush border)
  - Aminopeptidases
    - Cleave at N-terminal AA
  - Dipeptidases
    - Cleave dipeptides
  - Tripeptidase
    - Cleave tripeptides
    - (Enterokinase or Enteropeptidase)
      - Trypsinogen \(\rightarrow\) trypsin
      - Trypsin then activates all the other enzymes
Protein Digestion

- Proteins are broken down to
  - Tripeptides
  - Dipeptides
  - Free amino acids
Free Amino Acid Absorption

- Free amino acids
  - Carrier systems
    - Neutral AA
    - Basic AA
    - Acidic AA
    - Imino acids
  - Entrance of some AA is via active transport
    - Requires energy
## Amino Acid Transporters - Brush Border Membrane

<table>
<thead>
<tr>
<th>Transport system</th>
<th>Energy required</th>
<th>Substrates carried</th>
</tr>
</thead>
<tbody>
<tr>
<td>L, B, IMINO</td>
<td>No, Yes</td>
<td>Leu, other neutral Phe, Tyr, Trp, Ile, Leu, Val Pro, Gly Basic amino acids Most neutral and basic</td>
</tr>
<tr>
<td>y⁺, B⁰⁺, b⁰⁺</td>
<td>Yes, No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Peptide Absorption

- Form in which the majority of protein is absorbed
- More rapid than absorption of free amino acids
- Active transport
  - Energy required
- Metabolized into free amino acids in enterocyte
- Only free amino acids absorbed into blood
Absorption of Intact Proteins

• In Newborns
  - First 24 hours after birth
  - Immunoglobulins get absorbed
    • Passive immunity

• In Adults
  - By Paracellular routes
    • Tight junctions between cells
  - By Intracellular routes
    • Endocytosis
    • Pinocytosis

It has little nutritional significance...
- Affects health (allergies and passive immunity)
Protein Transport in the Blood

- Amino acids diffuse across the basolateral membrane
  - Enterocytes → portal blood → liver → tissues
  - Transported mostly as free amino acids
- Liver
  - Breakdown of amino acids
  - Synthesis of non-essential amino acids
Cystinuria is a disorder of the proximal tubule’s reabsorption of filtered cystine and dibasic amino acids (lysine, ornithine, arginine).

The inability to reabsorb cystine leads to accumulation and subsequent precipitation of stones of cystine in the urinary tract.
Catabolism of Amino acids
• Breakdown of Amino acid.
• Produce CO2 and NH3
• NH3 need to be detoxify
  - Peripheral detoxification
  - Peripheral to liver transport
  - In Liver detoxification
Peripheral detoxification

• First step is the removal of the α-amino group
  - by enzymes - **Amino-transferases** or **Transaminases**.
Alanine Amino-transferase
Alanine Transaminase (ALT)
Glutamate Pyruvate Transaminase (GPT)

Alanine + Alpha Ketoglutarate → Pyruvate + Glutamate
Aspartate Amino-transferase
Aspartate Transaminase (AST)
Glutamate Oxaloacetate Transaminase (GOT)

Aspartate  +  Alpha Ketoglutarate  $\rightleftharpoons$  Oxaloacetate  +  Glutamate
Transamination Reaction

- The amino group is transferred to α-ketoglutarate to make glutamate.
- Formation of Non-essential amino acid
- Formation substrate of Gluconeogenesis
- Detoxification of amide group
Glutamate + NADP⁺ + H₂O → α-Ketoglutarate + NADPH + H⁺ + NH₃
Glutamine transports NH$_3$ in the bloodstream

- Glutamate accepts the NH$_3$ by the action of Glutamine Synthetase.
- Glutamine transport to ammonia from periphery to liver
Urea

- If Ammonia is not used for production of new amino acids or other nitrogenous compounds, amino groups are transferred to the liver and converted to urea.
- Urea is produced in the cytosol via the urea cycle.
- Almost all urea is produced in the liver.
- Than Urea excreted in the urine.
The Urea Cycle

• The first two steps = mitochondrion.

• Remaining three = cytosol.
What can be use of this product?
Urea Cycle

- Glutamic acid
  - Oxidative deamination
  - NH$_3$ + CO$_2$
  - 2 ATP → 2 ADP

- Alpha-ketoglutaric acid
  - Carbamyl phosphate
  - Ornithine
  - Citrulline

- Arginine
  - ATP
  - Argininosuccinic acid
  - Oxaloacetic acid
  - Malic acid
  - Fumaric acid

Citric Acid Cycle

C. Ophardt, c. 2003
The enzymes catalyzing the urea cycle reactions

1. Ornithine transcarbamoylase
2. Argininosuccinate synthetase
3. Argininosuccinase
4. Arginase
The Urea Cycle and TCA Cycle are interconnected

- Cytosolic Isozymes of
  - Fumarase
  - Malate dehydrogenase.
- Malate enter in Mitochondria
- Than enter into the TCA cycle.
Regulation of the Urea Cycle

- Within an individual the movement of nitrogen through the cycle depends on diet.

- Changes in diet will only affect urea cycle activity over the long term.
Regulation of the Urea Cycle

- Short term
  - Carbamoyl Phosphate Synthetase.
  - Allosteric regulation
  - N-acetylglutamate activates CPS-1
  - Arginine activates N-acetylglutamate synthase,
Energetic cost of The Urea Cycle

\[ 2\text{NH}_4^+ + \text{HCO}_3^- + 3\text{ATP} + \text{H}_2\text{O} \rightarrow \text{urea} + 2\text{ADP} + 4\text{P}_i + \text{AMP} + 2\text{H}^+ \]

• However, through linkage of the pathways the toll is not so bad. Some NADH is produced which regains about 2.5 ATP form respiration.
Hereditary deficiency of any of the Urea Cycle enzymes leads to hyperammononemia - elevated [ammonia] in blood.

Total lack of any Urea Cycle enzyme is lethal.

Elevated ammonia is toxic, especially to the brain.

If not treated immediately after birth, severe mental retardation results.
Glutamine → NH₃ → Glutamine Synthase → Glutamate → Glutamate dehydrogenase → NH₃ → GABA

Alpha Keto glutarate

Citric Acid Cycle
Mechanisms for toxicity of high Ammonia

1. High [NH$_3$] would drive Glutamine Synthase:
   \[
   \text{glutamate + ATP + NH}_3 \rightarrow \text{glutamine + ADP + P}_i
   \]
   This would deplete glutamate – a neurotransmitter & precursor for synthesis of the neurotransmitter GABA.

2. Depletion of glutamate & high ammonia level would drive Glutamate Dehydrogenase reaction to reverse:
   \[
   \text{glutamate + NAD(P)$^+$} \leftarrow \alpha\text{-ketoglutarate + NAD(P)H + NH}_4^+
   \]
   The resulting depletion of \(\alpha\)-ketoglutarate, an essential Krebs Cycle intermediate, could impair energy metabolism in the brain.
Mechanisms for toxicity of high Ammonia

3. Due to high ammonia, conc. of Glutamine remains high in brain cell. Glutamine is co-transported outside from brain cell with tryptophan influx.

So, More Tryptophan get accumulated in brain cell if more glutamine goes out.

From accumulated Tryptophan, Serotonin synthesis & that have depressive effect on neurons.
Treatment of deficiency of Urea Cycle enzymes (depends on which enzyme is deficient):

- **limiting protein intake** to the amount barely adequate to supply amino acids for growth, while adding to the diet the \(\alpha\)-keto acid analogs of essential amino acids.

- **Liver transplantation** has also been used, since liver is the organ that carries out Urea Cycle.
One-carbon Transfer Reactions

- Cofactors for one-carbon transfer reactions in amino acid degradation.

- Tetrahydrofolate (H$_4$ folate) - Transfers carbon in intermediate oxidation states, sometimes methyl.

- S-adenosylmethionine (SAM or adoMet) - Transfers carbon as methyl groups.