Amino Acid Metabolism I
• The two major pathways of N acquisition
• The fate of ammonium
• Glutamine Synthetase
• Metabolic degradation of amino acids
• The synthesis of nonessential amino acids
• Specialized products derived from amino acids
Major Pathways for N Acquisition

• All biological compounds contain N in a reduced form
• The principal inorganic forms of N are in an oxidized state
• Thus, N acquisition must involve reduction of the oxidized forms ($N_2$ and $NO_3^-$) to $NH_4^+$
• Nearly all of this is in microorganisms and green plants. Animals gain N through diet.
The Nitrogen Cycle

Aerobic

- Nitrate respiration (dissimilation)
- Nitrification
- Nitrate
- NO₂⁻
- NO
- Denitrification
- N₂O
- N₂
- Nitrogen fixation
- NH₄⁺
- NO₂

Anaerobic

- Organic N

- Assimilation
Overview of N Acquisition

Nitrogen assimilation and nitrogen fixation

- Nitrate assimilation occurs in two steps: $2e^-$ reduction of nitrate to nitrite and $6e^-$ reduction of nitrite to ammonium
- Nitrate assimilation accounts for 99% of N acquisition by the biosphere
- Nitrogen fixation involves reduction of $N_2$ in prokaryotes by *nitrogenase*
Enzymology of N fixation

Only occurs in certain prokaryotes

- *Rhizobia* fix nitrogen in symbiotic association with leguminous plants
- *Rhizobia* fix N for the plant and plant provides *Rhizobia* with carbon substrates

All nitrogen fixing systems appear to be identical. They require *nitrogenase*, a *reductant* (reduced ferredoxin), ATP, O-free conditions and regulatory controls (ADP inhibits and NH$_4^+$ inhibits)
Nitrogenase Complex

Two protein components: nitrogenase reductase and nitrogenase

- Nitrogenase reductase is a 60 kD homodimer with a single 4Fe-4S cluster
- Very oxygen-sensitive
- Binds Mg ATP
- 4 ATP required per pair of electrons transferred
- Reduction of $N_2$ to $2 \text{NH}_3 + \text{H}_2$ requires 4 pairs of electrons, so 16 ATP are consumed per $N_2$
Ammonium Assimilation

Two principal pathways

- Principal route: GDH/GS in organisms rich in N (both steps assimilate N)

\[
\text{(a) } \text{NH}_4^+ + \alpha\text{-Ketoglutarate} + \text{NADPH} \xrightarrow{\text{GDH}} \text{Glutamate} + \text{NADP}^+ + \text{H}_2\text{O}
\]

\[
\text{(b) } \text{Glutamate} + \text{NH}_4^+ + \text{ATP} \xrightarrow{\text{GS}} \text{Glutamine} + \text{ADP} + \text{P}_i
\]

\[
\text{SUM: } 2 \text{NH}_4^+ + \alpha\text{-Ketoglutarate} + \text{NADPH} + \text{ATP} \rightarrow \text{Glutamine} + \text{NADP}^+ + \text{ADP} + \text{P}_i + \text{H}_2\text{O}
\]
• **Secondary route**: GS/GOGAT in organisms confronting N limitation

• GOGAT is *glutamate synthase* or *glutamate:oxo-glutarate amino transferase*

\[
\begin{align*}
(a) \quad 2 \text{NH}_4^+ + 2 \text{ATP} + 2 \text{Glutamate} & \xrightarrow{\text{GS}} 2 \text{Glutamine} + 2 \text{ADP} + 2 \text{P}_i \\
(b) \quad \text{NADPH} + \alpha\text{-Ketoglutarate} + \text{Glutamine} & \xrightarrow{\text{GOGAT}} 2 \text{Glutamate} + \text{NADP}^+ \\
\text{SUM:} \quad 2 \text{NH}_4^+ + \alpha\text{-Ketoglutarate} + \text{NADPH} + 2 \text{ATP} & \rightarrow \text{Glutamine} + \text{NADP}^+ + 2 \text{ADP} + 2 \text{P}_i
\end{align*}
\]
Enzymes cleaving the peptide chain

- **Endopeptidases** – hydrolyse the peptide bond inside a chain
  - **Pepsin** (pH 1.5 – 2.5) – peptide bond derived from Tyr, Phe, bonds between Leu and Glu
  - **Trypsin** (pH 7.5 – 8.5) – bonds between Lys a Arg
  - **Chymotrypsin** (pH 7.5 – 8.5) – bonds between Phe a Tyr

- **Exopeptidases** – split the peptide bond at the end of a protein molecule
  - **aminopeptidase**,  
  - **carboxypeptidases**,  
  - **dipeptidases**
Amino acid metabolism

- **BODY PROTEINS**
  - Proteosynthesis
  - Degradation

- **DIETARY PROTEINS**
  - Digestion
  - Transamination

- **GLYCOLYSIS / KREBS CYCLE**
  - Conversion (Carbon skeleton)

- **AMINO ACIDS**
  - UREA
  - NH₃

- **NONPROTEIN DERIVATIVES**
  - Porphyrins
  - Purines
  - Pyrimidines
  - Neurotransmitters
  - Hormones
  - Komplex lipids
  - Aminosugars

- **GLUCOSE**
- **CO₂**
- **KETONBODIES**

Conversion (Carbon skeleton)
Amino acid metabolism

Proteins

Amino acids

NH₃

Nucleotides

Pyruvate

Acetyl CoA

Citric acid cycle

Amino acids supply fuel to the citric acid cycle via several pathways. Used in biosynthesis of nucleotides

Reduced electron carriers (NADH, FADH₂)
Classification of proteinogenic AAs - metabolic point of view

1) **biosynthesis in a human body**
   - nonessential (are synthesized)
   - essential (must be present in a diet)

2) **degradation within cells**
   - glucogenic (Glc can be formed from their carbon skeleton)
   - ketogenic (= AAs degraded to acetyl-CoA)
Essential Amino Acids in Humans

• Required in diet
• Humans incapable of forming requisite carbon skeleton

• Arginine*
• Histidine*
• Isoleucine
• Leucine
• Valine
• Lysine
• Methionine
• Threonine
• Phenylalanine
• Tryptophan

* Essential in children, not in adults
Essential amino acids

1) branched chain: Val, Leu, Ile
2) aromatic: Phe (→ Tyr), Trp
3) basic: His, Arg, Lys
4) sulfur-containing: Met (→ Cys)
5) other: Thr
Non-Essential Amino Acids in Humans

- Not required in diet
- Can be formed from α-keto acids by transamination and subsequent reactions

- Alanine
- Asparagine
- Aspartate
- Glutamate
- Glutamine

- Glycine
- Proline
- Serine
- Cysteine (from Met*)
- Tyrosine (from Phe*)

* Essential amino acids
Degradation of Amino Acids

The 20 amino acids are degraded to produce (mostly) TCA intermediates.

- Know which are glucogenic
- Know which are ketogenic
- Know which are glucogenic and ketogenic
- Know which are purely ketogenic
Glucogenic Amino Acids

- Metabolized to $\alpha$-ketoglutarate, pyruvate, oxaloacetate, fumarate, or succinyl CoA

- Aspartate
- Asparagine
- Arginine
- Phenylalanine
- Tyrosine
- Isoleucine
- Methionine
- Valine
- Glutamine
- Glutamate
- Proline
- Histidine
- Alanine
- Serine
- Cysteine
- Glycine
- Threonine
- Tryptophan
Ketogenic Amino Acids

• Metabolized to acetyl CoA or acetoacetate

- Isoleucine
- Leucine
- Threonine
- Tryptophan

- Lysine
- Phenylalanine
- Tyrosine
General reactions of amino acids are transamination and deamination of α-amino group.

- **Deamination** leads to an α-keto acid.
- **Transamination** results in an α-keto acid and an amino acid.
- **Decarboxylation** produces a biologically active amine.
Degradation of amino acids

DECARBOXYLATION

R-CH-COOH

R-CH2-NH2

R-COOH

R-CO-COOH

FAD

FADH2

NAD

NADH

H2O

NH3

R-C-COOH

R-CO-COOH

R-CH2-COOH

R-CH2-COOH

R-CO-COOH

R-CO-COOH

aerobic decarboxylation

aerobic deamination

transamination

reduced deamination
Bazic steps in degradation of amino acids

• **Deamination** (elimination of amino group) shift amino group to ammonia or amino group of aspartate

• Build in atoms of N ammonia and aspartate to urea for elimination from organism – **urea cycle**

• Change the $\alpha$-oxoacids (carbons skeleton of amino acids after deamination) to standard metabolic intermediates
Deamination of AA

- **Transamination** (1. NH$_2$ group of AA is transferred to the enzyme - PLP and form oxoacid and aminated enzyme, 2. Amino group is transferred to ketonic acceptor (e.g. 2-oxoglutarate, oxalacetate, pyruvate), forms new aminoacid (e.g. glutamate, aspartate, alanine) and native forms of enzyme)

- **Oxidative deamination** (conversion glutamate by glutamate dehydrogenase and coenzymes NAD(P)$^+$ in mitochondria $\rightarrow \alpha$-iminoglutarate, which is hydrolysed to 2-oxoglutarate and ammonia)
1. Step - transfer $\alpha$-NH$_2$ on PLP: transamination(1), tautomer (2), hydrolyse (3) and transfer NH$_2$ on the ketogenic acceptor (4)

Mechanism of transamination
Transamination

Glutamate + α-Keto acid \rightarrow α-KG + α-Amino acid

Glutamate + Oxaloacetate \rightarrow α-KG + Aspartate
Clinicaly important transaminases

ALT

*alanine-α-ketoglutarate transferase*
Clinical marker for irreversibile liver damage

AST

*aspartate-α-ketoglutarate transferase*
Clinical marker for irreversibile myocardial damage
Essential (conditionally essential) nonessential amino acids

**essential:** Val, Leu, Ile, Thr, Phe, Trp, His, Arg, Lys, Met

**noness.:** Gly, Ala, Pro, Ser, Tyr, Asn, Gln, Asp, Glu, Cys

AAs ~ organically bound nitrogen

dietary proteins

body proteins

dev novo biosynthesis

proteosynthesis

AAs pool

N-compound synthesis

degradation (E, Glc, fat)
The Fate of Ammonium
Three major reactions in all cells

- **Carbamoyl-phosphate synthetase I**
  - two ATP required - one to activate bicarb, one to phosphorylate carbamate

- **Glutamate dehydrogenase**
  - reductive amination of alpha-ketoglutarate to form glutamate

- **Glutamine synthetase**
  - ATP-dependent amidation of gamma-carboxyl of glutamate to glutamine
Glutamate dehydrogenase

\[ \text{NH}_4^+ + \alpha\text{-ketoglutarate} \rightarrow \text{glutamate} \]

\[ \text{NADPH} + \text{H}^+ \rightarrow \text{NADP}^+ \]

\[ \text{NADH} + \text{H}^+ \rightarrow \text{NAD}^+ \]
Glutamine synthetase
The Urea Cycle I

• N and C in the guanidino group of Arg come from $\text{NH}_4^+$, $\text{HCO}_3^-$ (carbamoyl-P), and the $\alpha$-NH$_2$ of Glu and Asp

• Breakdown of Arg in the urea cycle releases two N and one C as urea
Urea (ornithine) cycle

= detoxification pathway (NH$_3$ is toxic for brain)

- proceeds only in the liver
- is localized in mitochondria / cytoplasm
- carbamoyl phosphate synthetase I (= mitoch.)
- can acidify the organism (consumes HCO$_3^-$)
- needs energy (3 ATP, but 4 energy rich bonds)
- is connected with citrate cycle through fumarate
- urea is end product of $-\text{NH}_2$ metabolism ($\rightarrow$ urine)
The Urea Cycle II enzymes

1. Carbamoylphosphate synthase
2. Ornithine-carbamoyltransferase
3. Argininosuccinate synthetase
4. Argininosuccinate lyase
5. Arginase
Regulation of urea cycle

**allosteric regulation** + enzyme induction by protein rich diet or by metabolic changes during starvation

<table>
<thead>
<tr>
<th><strong>regulatory enzyme</strong></th>
<th><strong>activation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>carbamoyl phosphate synthetase I (mitochondrial)</td>
<td>• N-acetylglutamate</td>
</tr>
<tr>
<td>N-acetylglutamate synthetase</td>
<td>• arginine</td>
</tr>
</tbody>
</table>

*Urea synthesis is inhibited by acidosis – HCO$_3^-$ is saved*
# Inherited defect in the urea cycle

<table>
<thead>
<tr>
<th>Disease</th>
<th>Defective enzyme</th>
<th>Product accumulated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperammonemia type I</td>
<td>CPS I</td>
<td>Ammonia, Gln, Ala</td>
</tr>
<tr>
<td>Hyperammonemia type II</td>
<td>Ornithine transcarbamoylase</td>
<td>Ammonia, Gln, orotic acid</td>
</tr>
<tr>
<td>Citrullinemia</td>
<td>Argininosuccinate synthetase</td>
<td>Citrulline</td>
</tr>
<tr>
<td>Argininosuccinic aciduria</td>
<td>Argininosuccinate lyase</td>
<td>Argininosuccinate</td>
</tr>
<tr>
<td>Argininemia</td>
<td>Arginase</td>
<td>Arg</td>
</tr>
</tbody>
</table>
Degradation of C-skeletons of AA

20 amino acids are degraded to seven products which are intermediates in central pathways of metabolism

- N – transamination, deamination and urea
- C-skeletons – sugars (glukoneogenesis) and lipids (synthese of FA)
- The AA can be classified into two major classes: **glucogenic** (pyruvate, oxaloacetate, fumarate, succinyl-CoA, \( \alpha \)-ketoglutarate) and **ketogenic** (acetyl-CoA, acetoacetyl-CoA) with a few being both keogenic and glucogenic
Degradation of amino acids (AAs)

1) -NH$_2$ group removing from AA

2) detoxification of the amino group

3) metabolism of carbon skeleton of AA - 7 products
7 degradation products of AAs

1. pyruvate ← Gly, Ala, Ser, Thr, Cys, Trp
2. oxaloacetate ← Asp, Asn
3. $\alpha$-ketoglutarate ← Glu, Gln, Pro, Arg, His
4. succinyl-CoA ← Val, Ile, Met, Thr
5. fumarate ← Phe, Tyr
6. acetyl-CoA ← Ile
7. acetoacetyl-CoA ← Lys, Leu, Phe, Tyr, Trp

(glucogenic AAs)
(ketogenic AAs)
Glucogenic (14)

- Ala
- Ser
- Cys
- Gly
- Thr
- Trp

Ketogenic (2 – Leu, Lys)

- Leu
- Lys
- Phe
- Tyr
- Trp

Both: Ile, Phe, Tyr, Trp
# Amino acid transport systems

<table>
<thead>
<tr>
<th>Transport system</th>
<th>Amino acids transported</th>
<th>Genetic disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neutral AAs</td>
<td>Ala, Gly, Ser, Thr, Val, Leu, Ile, Phe, Tyr, Trp, His, Cys, Met, citrulline</td>
<td>Hartnup disease</td>
</tr>
<tr>
<td>Acidic AAs</td>
<td>Glu, Asp</td>
<td>Dicarboxylic aminoaciduria</td>
</tr>
<tr>
<td>Dibasic AAs</td>
<td>Lys, Arg, cystine, ornithine</td>
<td>Cystinuria</td>
</tr>
<tr>
<td>Imino acids and glycine</td>
<td>Pro, OH-Pro, Gly</td>
<td>Joseph´s syndrome</td>
</tr>
</tbody>
</table>
Genetic defect in AAs metabolism

<table>
<thead>
<tr>
<th>Disease</th>
<th>Deficite in enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenylketonuria</td>
<td>Phenylalaninehydroxylase, cofaktor H₄biopterine</td>
</tr>
<tr>
<td>Thyrozinemia</td>
<td>different enzymes, degradation of Tyr</td>
</tr>
<tr>
<td>Albinisms</td>
<td>Thyrozinase in melanocytés</td>
</tr>
<tr>
<td>Homocystinuria</td>
<td>Cystatiónsyntetáza</td>
</tr>
</tbody>
</table>
Fate of amino nitrogen derived from AAs

**a) in extrahepatic tissues**

- transamination (forms mainly Ala and Glu + 2-oxoacids)
- deamination (only some AAs: Ser, Thr, His; releases NH\(_3\))
- amidation Glu + NH\(_3\) → Gln (needs ATP)

**b) in the liver**

- see a)
- oxidative deamination of Glu (forms \(\alpha\)-ketoGlt + NH\(_3\))

enzyme: glutamate dehydrogenase
Glucose-alanine cycle

Liver

Muscle cell

Blood

Gluconeogenesis

Urea

Kidneys

Glycolysis

O_2

ATP

NAD^+

NADH

Pyruvate

Alanine

ATP

Glucose
Ammonia transport

MOST TISSUES

Glutamate

$\text{NH}_4^+$

ATP

$\text{H}_2\text{O}, \text{ADP, P}_i$

Glutamine synthetase

Glutamine

LIVER

Glutamate

Glutaminase

$\text{NH}_4^+$

$\text{H}_2\text{O}$

Glutamine

$\text{Glu}$

$\alpha\text{KG}$

Pyruvate

Alanine

Alanine

Pyruvate

GLUCOSE–ALANINE CYCLE

Glucose

MUSCLE

Amino acids

Glutamate dehydrogenase

$\text{NH}_4^+$

$\text{Glu}$

$\alpha\text{KG}$

$\text{Glu}$

Glucose
Transport and detoxification of amino nitrogen

**SUMMARY**

- Aminotransferases $\rightarrow$ glutamate or alanine
- Glutamine synthetase $\rightarrow$ glutamine
- Glutaminase $\rightarrow$ glutamate + NH$_4^+$
- Glutamate dehydrogenase $\rightarrow$ 2-oxoglutarate + NH$_4^+$
- **Liver:** Urea cycle $\rightarrow$ urea $\rightarrow$ urine
- **Kidneys:** Glutaminase $\rightarrow$ glutamate + NH$_4^+$
Amino nitrogen released from carbon skeletons of AAs can be transported in blood as

a) NH$_4^+$
b) alanine
c) glutamine
d) urea
Detoxification of ammonia in a human body includes:

a) urea cycle proceeding only in the liver

b) cleavage of glutamine in the liver and the kidneys

c) consumption of energy in a form of ATP

d) formation of ornithine from citrulline and carbamoyl phosphate