Biosynthesis of Amino Acids

Chem 454: Biochemistry II
University of Wisconsin-Eau Claire
Biosynthesis of Amino Acids
Biosynthetic pathways for amino acids, nucleotides and lipids are very old.

Biosynthetic (anabolic) pathways share common intermediates with the degradative (catabolic) pathways.

The amino acids are the building blocks for proteins and other nitrogen-containing compounds.
Introduction

- **Nitrogen Fixation**
  - Reducing atmospheric $N_2$ to $NH_3$

- **Amino acid biosynthesis pathways**

- **Regulation of amino acid biosynthesis.**

- **Amino acids as precursors to other biological molecules.**
  - *e.g.*, Nucleotides and porphoryns
Introduction

Nitrogen fixation is carried out by a few select anaerobic microorganisms.

The carbon backbones for amino acids come from glycolysis, the citric acid cycle and the pentose phosphate pathway.

The L-stereochemistry is enforced by transamination of α-keto acids.
1. Nitrogen Fixation

Microorganisms use ATP and ferredoxin to reduce atmospheric nitrogen to ammonia.

- 60% of nitrogen fixation is done by these microorganisms
- 15% of nitrogen fixation is done by lighting and UV radiation.
- 25% by industrial processes
  - Fritz Haber (500°C, 300 atm)
1. Nitrogen Fixation

Enzyme has both a reductase and a nitrogenase activity.

Electrons from reduced ferredoxin

ATP → ADP + Pi

Reductase (Fe protein)

Nitrogenase (MoFe protein)

N₂ → NH₃
1.1 The Reductase (Fe protein)

Contains a 4Fe-4S center

- Hydrolysis of ATP causes a conformational change that aids the transfer of the electrons to the nitrogenase domain (MoFe protein)
1.1 The Nitrogenase (MoFe Protein)

The nitrogenase component is an $\alpha_2\beta_2$ tetramer (240 kD)
- Electrons enter the P-cluster
1.1 The Nitrogenase (MoFe Protein)

An Iron-Molybdenum cofactor for the nitrogenase binds and reduces the atmospheric nitrogen.
1.2 Assimilation of Ammonium Ion

The ammonium ion is assimilated into an amino acid through glutamate and glutamine.

Most amino acids obtain their α-amino group from glutamate by transamination.

The sidechain nitrogen of glutamine is the nitrogen source for the sidechain nitrogens of tryptophan and histidine.
1.2 Assimilation of Ammonium Ion

Glutamate dehydrogenase

\[
\text{NH}_4^+ + \alpha\text{-ketoglutarate} + \text{NADPH} + \text{H}^+ \rightleftharpoons \text{glutamate} + \text{NADP}^+ + \text{H}_2\text{O}
\]

- \(\alpha\text{-Ketoglutarate}\)
- \(\text{Glutamate}\)
1.2 Assimilation of Ammonium Ion

Glutamine synthetase

\[ \text{NH}_4^+ + \text{glutamate} + \text{ATP} \rightleftharpoons \text{glutamine} + \text{ADP} + P_i \]
The biosynthetic pathways can be grouped into families:

- **Oxaloacetate**
  - Aspartate
    - Asparagine
    - Methionine
    - Threonine
    - Lysine
  - Isoleucine
    - Alanine
    - Valine
    - Leucine
  - Pyruvate
    - Phosphoenolpyruvate
      - Erythrose 4-phosphate
        - Phenylalanine
        - Tyrosine
        - Tyrosine
  - α-Ketoglutarate
    - Glutamate
      - Glutamine
      - Proline
      - Arginine
  - 3-Phosphoglycerate
    - Serine
      - Cysteine
      - Glycine
  - Ribose 5-phosphate
    - Histidine
# 2.1 Essential Amino Acids

<table>
<thead>
<tr>
<th>Nonessential</th>
<th>Essential</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alanine</td>
<td>Histidine</td>
</tr>
<tr>
<td>Arginine</td>
<td>Isoleucine</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Leucine</td>
</tr>
<tr>
<td>Aspartate</td>
<td>Lysine</td>
</tr>
<tr>
<td>Cysteine</td>
<td>Methionine</td>
</tr>
<tr>
<td>Glutamate</td>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Glutamine</td>
<td>Threonine</td>
</tr>
<tr>
<td>Glycine</td>
<td>Tryptophan</td>
</tr>
<tr>
<td>Proline</td>
<td>Valine</td>
</tr>
<tr>
<td>Serine</td>
<td></td>
</tr>
<tr>
<td>Tyrosine</td>
<td></td>
</tr>
</tbody>
</table>

## TABLE 24.1 Basic set of 20 amino acids
2.1 Essential Amino Acids
2.2 Aspartate and Alanine

Transaminations:

\[
\text{Oxaloacetate} + \text{glutamate} \rightleftharpoons \text{aspartate} + \alpha\text{-ketoglutarate}
\]

\[
\text{Pyruvate} + \text{glutamate} \rightleftharpoons \text{alanine} + \alpha\text{-ketoglutarate}
\]
2.2 Aspartate and Alanine

Transaminations:
2.3 Asparagine

Amidation of aspartate
2.4 Proline and Arginine

Reduction of Glutamate
2.5 Serine and Glycine

Oxidation of 3-phosphoglycerate
2.5 Serine and Glycine

Serine transhydroxymethylase produces glycine from serine

$$\text{Serine} + \text{tetrahydrofolate} \rightarrow \text{glycine} + \text{methylene tetrahydrofolate} + \text{H}_2\text{O}$$
2.6 Tetrahydrofolate

In the news… pet food poison!
Pet Food Poison

Fluorouracil
↓
Fluorodeoxyuridylate (suicide inhibitor)

DUMP → dTMP
Thymidylate synthase

N^5, N^{10}-Methylene-tetrahydrofolate
Glycine
Serine
Tetrahydrofolate

Dihydrofolate

NADPH + H^+
Dihydrofolate reductase

Aminopterin and methotrexate (amethopterin)

Figure 25-13
Biochemistry, Sixth Edition
© 2007 W.H. Freeman and Company
# 2.5 Tetrahydrofolate

<table>
<thead>
<tr>
<th>Oxidation state</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most reduced ( = methanol)</td>
<td>–CH$_3$  Methyl</td>
</tr>
<tr>
<td>Intermediate ( = formaldehyde)</td>
<td>–CH$_2$– Methylene</td>
</tr>
<tr>
<td>Most oxidized ( = formic acid)</td>
<td>–CHO Formyl</td>
</tr>
<tr>
<td></td>
<td>–CHNH Formimino</td>
</tr>
<tr>
<td></td>
<td>–CH= Methenyl</td>
</tr>
</tbody>
</table>
2.5

Tetrahydrofolate
2.6 Methionine

Methylation of homocysteine

Homocysteine + $N^5$-Methyl-tetrahydrofolate → Methionine + Tetrahydrofolate
2.7 S-Adenosylmethionine (SAM)

Tetrahydrofolate does not have sufficient methyl transfer potential for many biosynthetic methylation reactions.

\[
\text{Methionine} + \text{ATP} \rightarrow \text{S-Adenosylmethionine (SAM)} + P_i + PP_i
\]
2.7 Activated Methyl Cycle

ATP $\rightarrow$ Methionine $\rightarrow$ S-Adenosyl-methionine $\rightarrow$ Active $\sim\text{CH}_3$

$\rightarrow$ S-Adenosyl-homocysteine $\rightarrow$ Homocysteine $\rightarrow$ $\text{H}_2\text{O}$

$\rightarrow$ $\text{CH}_3$
2.7 S-Adenosylmethionine

DNA methylation

Oligonucleotide target

Base to be methylated

S-Adenosylmethionine
2.10 Aromatic Amino Acids

Example of essential amino acid synthesis

- Involve Shikimate and Chorismate intermediates
2.10 Aromatic Amino Acids

Chorismate:
2.10 Tyrosine and Phenylalanine
2.10 Roundup

Glycophate inhibits the enzyme that converts 5-Enolpyruvylshikimate 3-phosphate to chorismate.

[Chemical structure of Glyphosate (Roundup)]
3. Regulation of Amino Acid Biosynthesis

Amino acid biosynthesis is regulated by feedback inhibition.

- The first committed step in a biosynthetic pathway is usually to the one that is regulated.

![Diagram showing amino acid biosynthesis pathway with inhibition]

**Inhibited by Z**
3. Regulation of Amino Acid Biosynthesis

Example: Serine biosynthesis

- 3-Phosphoglycerate dehydrogenase is inhibited by serine.

\[
\text{3-Phosphoglycerate} \xrightarrow{\text{NAD}^+ + \text{NADH}} \text{3-Phosphohydroxy-pyruvate} \xrightarrow{\text{Glutamate}, \alpha\text{-Keto-glutarate}} \text{3-Phosphoserine} \xrightarrow{\text{H}_2\text{O}, \text{P}_i} \text{Serine}
\]
3. Regulation of Amino Acid Biosynthesis

Example: Serine biosynthesis
- 3-Phosphoglycerate dehydrogenase
3.1 Regulation of Branched Pathways

Combination of feedback inhibition and activation
3.1 Regulation of Branched Pathways

The regulatory binding domain for threonine deaminase is similar to that found in 3-phosphoglycerate dehydrogenase.
3.1 Regulation of Branched Pathways

Enzyme multiplicity
- Example: Aspartokinase
  - Threonine
  - Methionine
  - Lysine

Aspartokinase domain

- Unregulated
- Threonine sensitive
- Lysine sensitive
3.1 Regulation of Branched Pathways

Cumulative feedback inhibition
- Example: Glutamine Synthetase

Glutamine is the source for nitrogen in the synthesis of:
- tryptophan
- histidine
- carbamoyl phosphate
- glucosamine 6-phosphate
- cytidine triphosphate
- adenosine monophosphate
3.1 Regulation of Branched Pathways

Cumulative feedback inhibition

- Example: Glutamine Synthetase
3.1 Regulation of Branched Pathways

**Cumulative feedback inhibition**

- Glutamine Synthetase activity is also modulated by enzymatic cascade
3.1 Regulation of Branched Pathways

Cumulative feedback inhibition
- Glutamine Synthetase activity is also modulated by and enzymatic cascade

![Diagram showing the regulation of branched pathways](image)

AT = adenylyl transferase

\[ P_D \text{ and } P_A = 2 \text{ forms of regulatory protein P} \]
3.1 Regulation of Branched Pathways

Cumulative feedback inhibition - More regulation!
- The regulatory protein P (P_A or P_D)
Amino acids are precursors for many biomolecules.

- Adenine
- Cytosine
- Sphingosine
- Histamine
- Thyroxine (Tetraiodothyronine)
- Epinephrine
- Serotonin
- Nicotinamide unit of NAD⁺
4. Amino Acid Derivatives

- Epinephrine: $X = \text{OH}, \ R = \text{CH}_3$
- Norepinephrine: $X = \text{OH}, \ R = \text{H}$
- Dopamine: $X = \text{H}, \ R = \text{H}$

Serotonin
(5-hydroxytryptamine)

$\text{OOC} - \text{CH}_2 - \text{CH}_2 - \text{CH}_2 - \text{NH}_3^+$

$\gamma$-Aminobutyric acid (GABA)

Histamine

$\text{CH}_2 - \text{CH}_2 - \text{NH}_3^+$
4. Amino Acid Derivatives

Glutamate

\[ \text{CO}_2 \]

\[ \begin{array}{c}
\text{glutamate} \\
\text{decarboxylase} \\
\text{(PLP dependent)}
\end{array} \]

\[ \text{γ-Aminobutyric acid (GABA)} \]

Histidine

\[ \text{CO}_2 \]

\[ \begin{array}{c}
\text{histidine} \\
\text{decarboxylase} \\
\text{(PLP dependent)}
\end{array} \]

Histamine

\[ \text{NH}_3^+ \]
4. Amino Acid Derivatives

Tryptophan → tryptophan hydroxylase → 5-Hydroxytryptophan → aromatic amino acid decarboxylase (PLP dependent) → Serotonin

Tetrahydrobiopterin + Dihydrobiopterin + O₂
4. Amino Acid Derivatives

Tyrosine

Dihydroxyphenylalanine (L-DOPA)

Dopamine

Norepinephrine

Epinephrine
4. Amino Acid Derivatives: some hallucinogenic and other amines
4. Amino Acid Derivatives

The Melanin Chemical Pathway

Tyrosinase

Tyrosine → Dopa → Dopaquinone + Cysteine

5-S-Cysteinyl-dopa → 2-S-Cysteinyl-dopa → Benzothiazine Intermediates → Pheomelanins

5,6-Dihydroxyindole → 2-carboxylic acid → Quinones → Eumelanins

Dopachrome Tautomerase

Leucodopachrome → Dopachrome

5,6-Dihydroxyindole → 5,6-Dihydroxyindole

"The Martyr of the Solway," 1871
by Sir John Everett Millais

"Nina" 2007
by Sara Shellenberger
4.1 Glutathione

Glutathione

- Sulfhydryl buffer and antioxidant

\[
\text{\textbf{\textgamma-Glutamate}} \quad \text{\textbf{Cysteine}} \quad \text{\textbf{Glycine}}
\]
Nitric oxide is a short-lived signal molecule.

- Formed from arginine

![Diagram of Nitric Oxide Formation](image)
4.3 Porphyrrins

Porphyrrins are synthesized from glycine and succinyl coenzyme A (PLP mechanism)
The mechanism of action of the PLP-dependent enzyme, δ-aminolevulinate synthase. The reaction steps are:

1. Transamination
2. PLP-stabilized carbanion formation
3. C-C bond formation
4. CoA elimination
5. Decarboxylation facilitated by the PLP-Schiff base
6. Transamination yielding ALA and regenerating the PLP-enzyme.

δ-Aminolevulinate (ALA)
4.3 Porphyrins
4.3 Porphyryns

[Diagram showing the pathway of porphyrin synthesis, including key enzymes and reactions such as ALA synthetase, Protoporphyrinogen III oxidase, and Ferrochelatase, leading to heme and finally to Hemoglobin.]
4.3 Porphyrins-degradation

http://www.umanitoba.ca/faculties/medicine/units/biochem/coursenotes/blanchaer_tutorials/Frank_II/urolbilenogen.html
4.3 Porphyrins-degradation

Heme

\[ 2 \text{O}_2 + \text{NADPH} \rightarrow \text{CO} + \text{H}_2\text{O} + \text{NADP}^+ \]

Fe\(^{3+}\)

Biliverdin

\[
\text{C05793}
\]

Stercobilin—brown

NADPH + H\(^+\)

\[
\text{C05794}
\]

urobilin—yellow
4.3 Porphyrins-disease states

Jaundice

• Neonatal
• Transfusion
• Hepatitis/cirrhosis

Porphyrias

• Porphobilinogen deaminase (hepatic porphyrias; acute intermittent porphyria)
• Uroporphyrinogen synthase, ferrochelatase (erythropoietic porphyrias)