Nitrogen Metabolism

Pratt and Cornely Chapter 18
Overview

• Nitrogen assimilation
• Amino acid biosynthesis
  — Nonessential aa
  — Essential aa
• Nucleotide biosynthesis
• Amino Acid Catabolism
• Urea Cycle

Juicy Steak Part 2
Nitrogen fixation

- Bacteria
- Nitrogenase
- Costly—16 ATP per $N_2$ molecule
Assimilation into Amino Acids

• In microorganisms/plants: assimilation of ammonia is key—synthesis of most amino acids
  – Glutamine synthetase incorporates amino group
  – Coupled to glutamine synthase: reductive amination of $\alpha$-ketoglutarate to glutamate
  – Glutamate distributes amino to new amino acids
Assimilation into Amino Acids

- In humans: acquire nitrogen in amino acids
  - No need for glutamine synthase
  - Glutamate distributes amino to new amino acids through transamination
  - Glutamine synthetase used to “mop up” ammonia
Transamination

- Transfers assimilated nitrogen into all other amino acids
- Requires PLP cofactor
1. The α-amino group of an amino acid attacks the enzyme–PLP Schiff base. This transamination reaction forms an amino acid–PLP Schiff base and releases the enzyme’s Lys ε-amino group.

2. The Lys amino group, acting as a base, removes the hydrogen from the substrate amino acid’s α carbon. The negative charge of the resulting carbanion is stabilized by the PLP group, which acts as an electron sink.

3. The protonated Lys residue, now acting as an acid, donates the proton to the PLP group, generating a ketimine. The molecular rearrangement resulting from the movement of an H atom is known as tautomerization.

4. Hydrolysis frees the α-keto acid and leaves the amino group bound to the PLP group.

5. Another α-keto acid enters the active site to reform a ketimine (this is the reverse of step 4).

6. Lysine-catalyzed tautomerization yields an amino acid–Schiff base (the reverse of steps 2 and 3).

7. In a transamination reaction, the e-amino group of the Lys residue displaces the amino acid and regenerates the enzyme–PLP Schiff base (the reverse of step 1).
Biosynthesis

• Dietary consideration
• Ambiguous
  – Stage of life (Arg)
  – Precursor (Tyr, Cys)
• Mechanism of biosynthesis can be grouped

<table>
<thead>
<tr>
<th>TABLE 18-1</th>
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<tbody>
<tr>
<td>Essential and Nonessential Amino Acids</td>
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<table>
<thead>
<tr>
<th>Essential</th>
<th>Nonessential</th>
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</thead>
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<tr>
<td>Histidine</td>
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<tr>
<td>Isoleucine</td>
<td>Arginine</td>
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<tr>
<td>Leucine</td>
<td>Asparagine</td>
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<tr>
<td>Lysine</td>
<td>Aspartate</td>
</tr>
<tr>
<td>Methionine</td>
<td>Cysteine</td>
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<tr>
<td>Phenylalanine</td>
<td>Glutamate</td>
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<td>Threonine</td>
<td>Glutamine</td>
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<td>Tryptophan</td>
<td>Glycine</td>
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<td>Valine</td>
<td>Proline</td>
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<td></td>
<td>Serine</td>
</tr>
<tr>
<td></td>
<td>Tyrosine</td>
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</tbody>
</table>
Amino Acid Biosynthesis

Oxaloacetate

Aspartate

Asparagine  Methionine  Threonine  Lysin

Isoleucine

Phosphoenolpyruvate + Erythrose 4-phosphate

Phenylalanine  Tyrosine  Tryptophan

Tyrosine

Pyruvate

Alanine  Valine  Leucine

α-Ketoglutarate

Glutamate

Glutamine  Proline  Arginine

Serine

Ribose 5-phosphate

Histidine

3-Phosphoglycerate

Cysteine  Glycine
Non-essential Amino Acid Biosynthesis

• Transamination
  – Pyruvate → alanine
  – Oxaloacetate → aspartate
  – α-ketoglutarate → glutamate

• Amidation
  – Glutamine (glutamine synthetase)
  – Asparagine (asparagine synthetase)
Glutamate Backbone

Proline

Glutamate

Arginine
Serine/Glycine

- 3-phosphoglycerate $\rightarrow$ Serine
- Serine $\rightarrow$ glycine
  - THF as a major one-carbon transfer vitamin
Figure 24.10
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Cysteine and Tyrosine

• Serine $\rightarrow$ cysteine by incorporating sulfur from homocysteine (Made from methionine)

\[
\text{Serine} \quad \text{Homocysteine} \quad \text{Cysteine} \quad \text{\(\alpha\)-Ketobutyrate}
\]

• Oxidation of Phe gives tyrosine

\[
\text{Phenylalanine} \quad \xrightarrow{\text{phenylalanine hydroxylase}} \quad \text{Tyrosine}
\]
Neurotransmitters

• Which amino acid?
Nucleotide Biosynthesis

- 5-PRPP
- **Purine:** base built onto ribose
  - Asp, Gly, Glu, THF, bicarbonate
- IMP produced
AMP/GMP Production

- Branched pathway
- AMP: amination
- GMP: oxidation
- Branch allows for reciprocal regulation
Pyrimidines

- Contrast
- Base made first, then attached to 5PRPP
- Not branched: UMP made to UTP then to CTP
Ribonucleotide Reductase

- Essential reaction: reduction to make dNDP
- Very difficult reaction
- Free radical
- Enzyme is oxidized in the process
  - Reduced by thioredoxin
  - In turn, thioredoxin reduced by NADPH
1. The Cys free radical (—S·) reacts with the ribose of the NDP substrate to create a radical at C3′.

2. An enzyme Cys SH group donates a proton to the oxygen at C2′.

3. The radical helps stabilize the carboxylation at C2′ formed by the loss of H₂O.

4. Transfer of a proton and electron reduces the cation and produces a disulfide bond between two enzyme Cys residues.

5. A second proton and electron transfer (the reverse of step 1) generates the deoxyribose group and regenerates the thyl radical.

6. The oxidized Cys groups of the enzyme are reduced in a disulfide exchange reaction with thioredoxin.

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Production of TMP

- dUTP must be converted to TMP quickly
- Methylene donated from THF by thymidylate synthase
- THF oxidized to DHF
- Chemotherapy: dUMP analog
Regenerating THF

- DHF must be reduced to THF by DHF reductase
- NADPH dependent
- Chemotherapy dtarget
  - DHF analogs such as methotrexate
Catabolism

- Salvage pathway through phosphorolysis
- Purines made into uric acid (waste)
- Pyrimidines broken down into catabolic intermediates

![Diagram of catabolism process](image-url)
Amino acid catabolism

Figure 23.1
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# Ketogenic vs. Glucogenic

## Table 18-2: Catabolic Fates of Amino Acids

<table>
<thead>
<tr>
<th>Glucogenic</th>
<th>Both Glucogenic and Ketogenic</th>
<th>Ketogenic</th>
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<tbody>
<tr>
<td>Alanine</td>
<td>Isoleucine</td>
<td>Leucine</td>
</tr>
<tr>
<td>Arginine</td>
<td>Phenylalanine</td>
<td>Lysine</td>
</tr>
<tr>
<td>Asparagine</td>
<td>Threonine</td>
<td></td>
</tr>
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<td></td>
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<tr>
<td>Cysteine</td>
<td>Tyrosine</td>
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<tr>
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<tr>
<td>Serine</td>
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<tr>
<td>Valine</td>
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</tbody>
</table>

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Problem 35

• The catabolic pathways for the 20 amino acids ary considerably, but all amino acids are degraded to one of seven metabolites: pyruvate, $\alpha$-ketoglutarate, succinyl-CoA, fumarate, oxaloacetate, acetyl CoA, are acetoacetate. What is the fate of each of these metabolites?
Amino Acid Degradation

- Transamination and deamination
- Then carbon chain is metabolized
- Examples:

![Chemical structures of amino acid degradation](image-url)
Pyruvate Producing

Serine \[\xrightarrow{NH_4^+} \text{Pyruvate}\]

Cysteine \[\xrightarrow{NH_4^+} \xrightarrow{H_2O, H_2S} \text{Pyruvate}\]
Glutamate Family

- 25% of dietary intake

Phosphoenolpyruvate $\rightarrow$ Pyruvate $\rightarrow$ Acetyl CoA $\leftrightarrow$ Acetoacetyl CoA

Oxaloacetate $\rightarrow$ Fumarate $\rightarrow$ Succinyl CoA $\rightarrow$ $\alpha$-Keto glutarate

Arginine, Glutamate, Glutamine, Histidine, Proline
Thr: Glucogenic and Ketogenic

- Gly—major source of methylene-THF
Branched Amino Acids

• Major energy source in muscle
• Steps of degradation
  – Transamination
  – Oxidative decarboxylation (Pyruvate DH)
  – Beta oxidation
• Valine: succinyl CoA
• Isoleucine: succinyl CoA and acetylCoA
• Leucine: acetyl CoA and ketone body
Problem 40

• Leucine is degraded to acetyl CoA and acetoacetate by a pathway whose first two seps are identical to those of valine degradation (Figure 18-11). The third step is the same as the first step of fatty acid oxidation. The fourth step involves an ATP-dependent carboxylation, the fifth step is a hydration, and the last step is a cleavage reaction to give products. Draw the intermediates of leucine degradation.
Degradation of branched-chain amino acids

\[
\begin{align*}
\text{Isoleucine} & \rightarrow \text{Valine} & \rightarrow \text{Leucine} \\
\text{Transamination (PLP)} & \rightarrow \text{α-Ketoglutarate} \\
1. \text{Oxidative Decarboxylation} & \rightarrow \text{Succinyl-CoA} \\
2. \text{Acyl-CoA Dehydrogenation} & \rightarrow \text{Fumarate} \\
3. \text{Hydration} & \rightarrow \text{5-Hydroxy-3-methylglutaryl-CoA (HMG-CoA)} \\
4. \text{Dehydrogenation} & \rightarrow \text{Malate} \\
5. \text{Thiolic cleavage} & \rightarrow \text{Acetoacetate} \\
& \rightarrow \text{Propionyl-CoA} & \rightarrow \text{Acetyl-CoA} & \rightarrow \text{Acetyl-CoA} \\
& \rightarrow \text{Acetoacetate} & \rightarrow \text{Acetyl-CoA} & \rightarrow \text{Acetyl-CoA}
\end{align*}
\]

\text{β-oxidation of fatty acids (cf Figure 16.15)}

\text{Citric acid cycle (cf Figure 13.3)}

FIGURE 16.14

Branched-chain amino acid oxidation, fatty acid β-oxidation, and the citric acid cycle share a common chemical strategy.
Aromatic Amino Acids

- Complicated
- First recognition of inborn errors of metabolism

Phenylalanine → Tyrosine → $p$-Hydroxyphenylpyruvate → Homogentisate → 4-Maleylacetoacetate

1. Missing in phenylketonuria: phenylalanine hydroxylase
2. Missing in alcaptonuria: homogentisate dioxygenase
Problem 51

• List all the reactions in this chapter that generate free ammonia.
Ammonia Processing

• Most tissues: glutamine synthetase “mops up”
  – glutamine sent through blood to liver
  – Deaminated in liver to give glutamic acid
    • glutaminase
Ammonia Processing

• Muscle: The alanine-glucose cycle
• Glutamate also accepts amino group from other amino acids
Glutamate Dehydrogenase

- Reversible reaction
- Grabs free ammonia and releases it in liver mitochondria

\[
\begin{align*}
\text{Glutamate} &\quad \text{NH}_3^+ \\
\text{NAD(P)H} + \text{H}^+ \\
\text{NAD(P)}^+ &\quad \text{H}_2\text{O} \quad \text{NH}_4^+ \\
\left[\text{\begin{tabular}{c}
\text{\(-OOC-CH_2-CH_2-C-\text{COO}^-
\end{tabular}}
\right] &\quad \text{\(-OOC-CH_2-CH_2-C-\text{COO}^-
\end{tabular}}
\right] \\
\text{\(-OOC-CH_2-CH_2-C-\text{COO}^-
\end{tabular}} &\quad \alpha\text{-Ketoglutarate}
\end{align*}
\]
Role of Liver Mitochondria

- Sequester toxic ammonia
- Make less toxic, execrable form
- Urea Cycle

\[
\begin{align*}
\text{bicarbonate} & \\
\text{ammonia} & \quad \text{(from glutamate)} \\
aspartate & \quad \text{(can be derived from glutamate via transamination)}
\end{align*}
\]
Carbamoyl phosphate

• **Cost of 2 ATP**
  – Phosphate leaving group

• **Activation of ammonia for**
  – Excretion
  – biosynthesis
Problem 52

• Which three mammalian enzymes can potentially “mop up” excess NH$_4^+$?
1. Ornithine transcarbamoylase produces citrulline by transferring a carbamoyl group to ornithine.

2. Argininosuccinate synthetase adds aspartate to citrulline in an ATP-dependent condensation reaction. The products of the reaction include AMP and PP\textsubscript{i}, which is subsequently hydrolyzed to 2 P\textsubscript{i}. Consequently, this step consumes 2 ATP equivalents.

3. Argininosuccinate releases fumarate (which represents the carbon skeleton of aspartate) from argininosuccinate.

4. Arginase hydrolyzes arginine to generate urea and regenerate ornithine for another round of the urea cycle.
Chemistry of Urea Cycle

- Catalytic ornithine
- Fumarate
- Urea = 4 ATP
Compartmentalization
Urea Cycle Regulation

- Carbamoyl phosphate synthetase
- Amino acid catabolism boosts acetyl CoA and glutamate levels
- Produces activator
Problem 55

• An inborn error of metabolism results in the deficiency of arginosuccinase. What could be added to the diet to boost urea production aid in ammonia secretion? (Argininosuccinate can be excreted.)
Solving Metabolic Problems

- Arginosuccinase deficiency
- Low protein diet
  - Minimize ammonium
- High arginine diet
  - Provide carrier
Nitrogen Flow Overview

Amino acid

α-Ketoglutarate

transaminase

Glutamate

NH₃

glutamate dehydrogenase

carbamoyl phosphate synthetase

Carbamoyl phosphate

Ornithine

Citrulline

Urea

Arginine

Argininosuccinate

Fumarate

Aspartate

Oxaloacetate

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Summary: Main Players

- Glutamate: in liver, receives nitrogen from AA, then ammonia is released in liver mitochondria
- Glutamine: ammonia transport; biosynthesis
- Alanine: ammonia transport
- Aspartate: nitrogen donor to urea
- Arginine: urea cycle