Amino Acid Metabolism II
Amino Acid Biosynthesis

• Plants and microorganisms can make all 20 amino acids and all other needed N metabolites.
• In these organisms, glutamate is the source of N, via transamination (aminotransferase) reactions.
• Mammals can make only 10 of the 20 aas.
• The others are classed as "essential" amino acids and must be obtained in the diet.
• All amino acids are grouped into families according to the intermediates that they are made from.
Biosynthese of nonessential amino acids

From 4 intermediates of metabolism:

- **2-oxoglutarate** (α-ketoglutarate family)
- **oxalacetate** (aspartate family)
- **pyruvate**
- **3-phosphoglycerate** (serine family)
The \( \alpha \)-Ketoglutarate Family

*Glu, Gln, Pro, Arg, and sometimes Lys*

- Glutamate is precursor
- Proline pathway need ATP and NAD(P)H as donor H\(^+\)
- Look at ornithine pathway to see the similarity to the proline pathway
\(\alpha\)-Ketoglutarate

Glutamate

Glutamine    Proline    Arginine

Pro is cyclized Glu
The Aspartate Family
Asp, Asn, Lys*, Met*, Thr*, Ile*

- Transamination of OAA gives Asp
- Amidation of Asp gives Asn
- Thr*, Met*, and Lys* are made from Asp
- β-Aspartyl semialdehyde and homoserine are branch points
- Note role of methionine in methylations via S-adenosylmethionine
Transamination of oxaloacetate

\[
\text{Oxaloacetate} + \text{Glutamate} \rightarrow \text{Aspartate} + \alpha\text{-Ketoglutarate}
\]
The Pyruvate Family

Ala, Val*, Leu*

- Transamination of pyruvate gives Ala
- Val is derived from pyruvate
- Note that Ile synthesis from Thr mimics Val synthesis from pyruvate
- Leu synthesis, like that of Ile and Val, begins with an $\alpha$-keto acid
- Transaminations from Glu complete each of these pathways
3-Phosphoglycerate Family
Ser, Gly, Cys

- 3-Phosphoglycerate dehydrogenase diverts 3-PG from glycolysis to AA paths
- Transamination by Glu gives 3-P-serine
- Phosphatase yields serine
- Serine hydroxymethylase (PLP) transfers the $\beta$-carbon of Ser to THF to make glycine
- A PLP-dependent enzyme makes Cys
3-Phosphoglycerate

Serine

Glycine  Cysteine
Aromatic Amino Acids
Phe*, Tyr, Trp*, His*

• Shikimate pathway yields Phe*, Tyr, Trp*
• Note the role of chorismate as a branch point in this pathway
• His* synthesis, like that of Trp*, shares metabolic intermediates with purine biosynthetic pathway
Coenzyme Q  
$p$-Hydroxybenzoate  
Vitamin K  
Folic acid  
$p$-Aminobenzoate (PABA)  
Anthranilate  
Tryptophan  
Chorismate  
Prephenate  
Phenylalanine  
Plastoquinone  
Vitamin E  
Tyrosine  
Lignin (a complex polymer of $C_9$ aromatic units)
The importance of chorismate
Synthesis of AAs in a human body
4 (5) substrates

1. oxaloacetate → Asp, Asn
2. α-ketoglutarate → Glu, Gln, Pro, (Arg)
3. pyruvate → Ala
4. 3-phosphoglycerate → Ser, Cys, Gly
5. Phe* → Tyr
Synthesis of AAs in a human body

**important reactions**

1. **transamination**
   - Pyr → Ala
   - OA → Asp
   - $\alpha$-ketoGlt → Glu

2. **amidation**
   - Asp → Asn
   - Glu → Gln

3. **synthesis from other amino acids**
   - Phe → Tyr
   - Ser → Gly
   - Glu → Pro
   - Met + Ser → Cys
1. Transamination reaction

REVERSIBLE

- **enzymes**: amino transferases
- **coenzyme**: pyridoxal phosphate (vit. B6)
Amino transferases important in medicine ("transaminases")

- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
2. amidation

„amidation“ of glutamate side chain carboxylic group of Glu is converted to amide group

GLUTAMINE

the most important transport form of amino nitrogen in blood

2. amidation
Synthesis of ASPARAGINE needs glutamine as –NH₂ group donor

(it is not ammonia as in the Gln synthesis)
3. synthesis AA from other AAs

- Synthesis Tyr from Phe*
- Biosynthesis Ser and Cys from Met*
Synthesis of Tyr from Phe*
Synthesis of Cys from Met* and Ser

Methionine synthase

Methionine

ATP, PPi + Pi

SAM

Homocysteine

THF

N5-methyl-THF

Cystathionine synthase

Cystathionine lyase

α-Ketobutyrate

Cystathionine

Serine
Some amino acids are used for synthesis of other N-compound:

1) Gln, Asp, Gly → purines, pyrimidines
2) Gly → porphyrines, creatine is found in muscle and brain tissue where it serves as a reservoir of high-energy phosphate groups (from Gly, Arg, Met)
3) Arg → NO nitric oxide – powerful vasodilator and neurotransmitter
4) Cys → taurine
Arginine as a precursor of creatinine

- **Arginine**
- **Glycine**
- **Ornithine**
- **Guanidoacetate**
- **S-Adenosylmethionine**
- **S-Adenosylhomocysteine**
- **Creatine**
- **Creatine Phosphate**
- **ATP**
- **ADP**
- **Creatine Phosphokinase**
- **Nonenzymatic**
- **In kidney**
- **In liver**
Arginine and NO
Synthesis of taurine from cysteine through cysteinesulfinic acid and then to hypotaurine.
Decarboxylation of AAs gives monoamines (biogenic amines)

1) Tyr → **catecholamines** (adrenaline, noradrenaline, dopamine)
2) Trp → **serotonin** (5-hydroxytryptamine)
3) His → **histamine**
4) Ser → **etanolamine** → choline → acetylcholine
5) Cys → **cysteamine**
   \[ \text{Asp} \rightarrow \beta\text{-alanine} \]
   \[ \text{Glu} \rightarrow \gamma\text{-aminobutyrate (GABA)} \]
Specialized products derived from AAs

- Carriers of single-carbon fragments in metabolism (biotin, SAM, THF)
- Neurotransmitters and hormones (catecholamines, serotonin, melatonin, histamin, GABA)
- Thyroid hormones ($T_3$ and $T_4$)
- Phorphyrin and heme metabolism
- Other specialised products (melanins, glutathione)
Carriers of single-carbon fragments in metabolism

- Biotin transfers carboxyl groups (pyruvate carboxylase, acety-CoA carboxylase, propionyl-CoA carboxylase)
- SAM – major carrier of methyl groups in metabolism
- THF transfers carbon atoms at all other oxidation states (methyl, hydroxymethyl, formyl, methenyl)
Formation of activated methionine

*S-adenosylmethionine (SAM)*

SAM is used as –CH₃ group donor in metabolic methylations
## Selected methylation reactions involving SAM

<table>
<thead>
<tr>
<th>Acceptor</th>
<th>Product</th>
<th>Metabolic pathway Synthesis of</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guanidoacetic acid</td>
<td>Creatine</td>
<td>Creatine</td>
</tr>
<tr>
<td>Phosphatidylethanolamine</td>
<td>Phosphatidylcholine</td>
<td>Phospholipid</td>
</tr>
<tr>
<td>rRNA, tRNA</td>
<td>Methylated RNA</td>
<td>RNA</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>Epinephrine</td>
<td>Catecholamine</td>
</tr>
<tr>
<td>Protein-bound lysine</td>
<td>Trimethyllysine</td>
<td>Carnitine</td>
</tr>
</tbody>
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Tetrahydrofolate - THF

- The major carrier of single-carbon atoms in metabolism
- Folic acid – the vitamin precursor of THF, is reduced to the active cofactor form in a reaction requiring the reduced form of NADPH
- Formiminoglutamate (FIGLU) is an intermediate in His degradation that donates a carbon atom to THF, resulting in 5-formimino-THF, which can be converted to methenyl-THF. A deficiency in folate prevents this reaction from occurring and results in the excretion of FIGLU in the urine
Reduction the folate to THF (pteroylglutamate acid)

2-amine-4-oxo-6-methylpterine  PABA
AAs- precursors of neurotransmitters and hormones

• Aromatic AAs are important precursors (Tyr, Trp)
• Catecholamines and thyroid hormones are derived from Tyr
• Serotonin and melatonin are derived from Trp
• Glu and His are precursors of other biogenic amines
Conversion of AAs to bogenic amines

Involves three types of reactions

1. decarboxylation
2. hydroxylation
3. SAM – dependent methylation
Biosynthesis of catecholamines
Dopamine, adrenaline and noradrenaline – aminoderivatives of catechol
Monoamine Oxidase (MAO)

MAO inhibitors (e.g., tranylcypromine) are useful in the treatment of depression.

Brain levels of dopamine and norepi.; also serotonin.

R=OH Vanillylmandelic acid
R=H Homovanillic acid
Tyramine

- Tyramine found naturally in several types of cheese; also beer and red wine.
- Tyramine intake can cause hypertensive crisis in persons taking a MAO inhibitor (↑norepi release)
Catechol-O-Methyl Transferase (COMT)

- COMT found in cytoplasm
- Terminates activity of catecholamines
- Catecholamine excretion products result from combined actions of MAO and COMT
- Inhibitors of COMT (e.g., tolcapone) useful in Parkinson’s disease
Homogentisic Acid Formation

Tyrosine

\[ \text{Transamination} \]

\[ \text{Homogentisate} \]

\[ \text{Homogentisate} \text{dioxygenase} \]

\[ \text{Cleavage of aromatic ring} \]

\[ \text{Deficient in alkaptonuria} \]

\[ \text{Homogentisate dioxygenase} \]

\[ \text{Cleavage of aromatic ring} \]

\[ \text{Fumarate + acetoacetate} \]

\[ \text{p-Hydroxyphenyl-pyruvate} \]

\[ \text{p-Hydroxyphenyl-pyruvate dioxygenase (ascorbate-dep.)} \]

\[ \text{O}_2 \]

\[ \text{CO}_2 \]
Melanin Formation

Melanin formed in skin (melanocytes), eyes and hair
In skin, protects against sunlight
Albinism: genetic deficiency of tyrosinase
Tryptophan Metabolism: Serotonin Formation

Tryptophan (Trp)

Indole ring

+ NH₃

CH₂CH₂CO₂⁻

Trphydroxylase

O₂

5-Hydroxy-tryptophan

Decarboxylase

+ NH₃

CH₂CH₂CO₂⁻

5-Hydroxy-tryptamine (5-HT)

Serotonin

CH₂CH₂NH₂
Serotonin

• Serotonin formed in:
  • Brain (neurotransmitter; regulation of sleep, mood, appetite)
  • Platelets (platelet aggregation, vasoconstriction)
  • Smooth muscle (contraction)
  • Gastrointestinal tract (enterochromaffin cells - major storage site)

• Drugs affecting serotonin actions used to treat:
  • Depression
    • Serotonin-selective reuptake inhibitors (SSRI)
  • Migraine
  • Schizophrenia
  • Obsessive-compulsive disorders
  • Chemotherapy-induced emesis

• Some hallucinogens (e.g., LSD) act as serotonin agonists
Serotonin Metabolism: 5-HIAA

Carcinoid tumors:
- Malignant GI tumor type
- Excretion of large amounts of 5-HIAA

Serotonin

\[
\text{CH}_2\text{CH}_2\text{NH}_2
\]

MAO

\[
\text{CH}_2\text{CHO}
\]

Dehydrogenase

5-Hydroxyindole acetic acid (5-HIAA) (Urine)
Melatonin:
- Formed principally in pineal gland
- Synthesis controlled by light, among other factors
- Induces skin lightening
- Suppresses ovarian function
- Possible use in sleep disorders
Tryptophan Metabolism: Biosynthesis of Nicotinic Acid

Tryptophan

\[
\begin{align*}
+ & \\
\text{NH}_3 & \\
\text{CH}_2\text{CHCO}_2^- & \\
\end{align*}
\]

Several steps

Nicotinic acid (Niacin)

\[
\begin{align*}
\text{CO}_2\text{H} & \\
\end{align*}
\]

Nicotinamide adenine dinucleotide (NAD)
Protein-bound Tyr – the precursor of thyroid hormones

- The thyroid gland produces two hormones: triiodothyronine (T₃) and tetraiodothyronine (T₄).
- Substrates – thyroglobulin (globular protein, 660 kDa), iodine, hydrogen peroxide.
- From 140 Tyr residues of thyroglobulin, about 20% are iiodinated with iiodideperoxidase: 2,5-diiodo-Tyr (T₃ and T₄ contain both residues).
- Cross-linking of iodinated Tyr residues and release of T₃ and T₄ from thyroglobulin by hydrolysis (proteolysis).
Biosynthese $T_3$ and $T_4$ in the thyroid gland
GABA - \( \gamma \)-aminobutyric acid

- Is formed by the release of the \( \alpha \)-carboxyl group of glutamate – by \textit{L-glutamate decarboxylase (PLP)}
- GABA is found in high concentrations in brain – inhibitory neurotransmitter
- Huntington’s disease – uncontrolled movement
GABA Formation

Drugs (e.g., benzodiazepines) that enhance the effects of GABA are useful in treating epilepsy.
Biosynthesis of histamine

- It is a chemical messenger involved in numerous cellular responses.
- Plays an important role in mediating allergic and inflammatory reactions.
- It is a powerful vasodilator.

Diagram: Histidine to Histamine conversion.

- Histidine → Histamine
- Histidine decarboxylase
- CO₂
- +NH₃

Chemical structures:
- Histidine: H₂N-CH₂-C-COÖ⁻
- Histamine: H₂N-CH₂-CH₂-N-CH₂-H
Other specialized products derived from AAs - glutathione

- Glutathione – tripeptide (Glu, Cys, Gly)- is the most abundant sulfur-containing compound in cells (about 5 mM)
- Non-ribosomal peptide biosynthesis
- Leads to reducing intracellular environment
- Few structural disulfides in intracellular proteins vs many in extracellular (antibodies, growth hormone, etc.)
- Involved in drug metabolism (conjugation to drug for feces/urine)
Glutathione (GSH, γ-glutamylcysteinylglycine)

Functions

1. Detoxification of toxic electrophilic xenobiotics

2. Preventing oxidative damage and hemolysis – it is essential in reducing hydrogen peroxide levels (peroxidase)

3. It plays an important role in eicosanoid synthesis (glutathion-S-transferase)
4. One of the most important functions is to maintain protein sulphydryl group in their reduced state

- normally, the reduced form (GSH) constitutes about 98% of the total glutathione pool

- (GSSG) is a oxidized glutathione

- glutathione reductase and reduced form of NADPH
γ-glutamyls cycle

γ-Glu-cys-gly → Cys-gly → γ-Glu-amino acid

Plasma membrane

Cys-gly + H₂O → γ-Glu-amino acid

Amino acid

γ-Glutamyl cyclotransferase

Glutathione

ADP + Pᵢ + H⁺ → ATP

Glutathione synthase

5-Oxoproline

ATP + 2 H₂O

5-Oxoprinase

Cys a Met

L-Cysteine

ADP + Pᵢ + H⁺ → ATP

γ-Glutamylcysteine synthase

L-Glutamate

SH

γ-Glutamylcysteine synthase

OOC-CH₂-CH₂-CH₂-COO⁻

NH₃

H₃N+—CH—COO⁻
Polyamines

- Spermidine and spermine found in virtually all procaryotic and eucaryotic cells
- DNA packaging (like histones) come from ornithine and methionine
  - Bind to nucleic acids
- Inhibition of biosynthetic pathway:
  - $\alpha$-Difluoromethyl-ornithine (DFMO) (Eflornithine) - inhibits ODC; used to treat *Pneumocystis carinii* infections
Synthesse of spermine

Omithine

CO₂

ODC

H₃N–(CH₂)₄–NH₃

Putrescine

SAM

5′-methylthioadenosine

H₃N–(CH₂)₄–NH₂–(CH₂)₃–NH₃

Spermidine

SAM

5′-methylthioadenosine

H₃N–(CH₂)₃–NH₂–(CH₂)₄–NH₂–(CH₂)₃–NH₃

Spermine
Polyamine Biosynthesis

Ornithine (from urea cycle) + NH$_3$ $\rightarrow$ Putrescine

Decarboxylated SAM $\rightarrow$ Spermidine synthase $\rightarrow$ Spermidine

5'-Methylthio-adenosine $\rightarrow$ Spermine synthase $\rightarrow$ Spermine

H$_3$NCH$_2$CH$_2$CH$_2$CHCO$_2$ -

Ornithine decarboxylase (ODC) (PLP-dep.)

H$_3$NCH$_2$CH$_2$CH$_2$CHCO$_2$ -

CO$_2$

H$_3$NCH$_2$CH$_2$CH$_2$CHCO$_2$ -

NH$_3$
Porphyrin and heme metabolism

**Heme** – prosthetic group for hemoglobin, myoglobin and cytochromes

- it is porphyrins with high affinity for binding metal ions
  – contain four pyrrole rings, which are linked together by single-carbon bridges

- all of the toms in porphyrins are derived from *Gly* and *succinyl-CoA*
Key steps in heme synthesis

- Condensation of succinyl-CoA with glycine + decarboxylation (PLP) – 5-aminolevulinic acid (ALA) in mitochondria – it is the rate-limiting step

- Two molecule of 5-aminolevulinate results in porphobilinogen (PBG) in cytosole – this reaction provides the pyrrole ring system that is used to assemble porphyrins– inhibition of PBG-syntase by Pb

- Formation of uroporphyrinogen III by condensation of 4 molecules of PBG

- Uroporphyrinogen III is converted to protoporphyrin IX through a series of decarboxylation and oxidation reaction – the final step in heme synthesis involves the introduction of Fe^{2+} into protoporphyrin IX ring system
Degradation of heme

1. Oxidizes one of the carbon bridges that connect the pyrrole rings – form linear green pigment tetrapyrrol **biliverdin**

2. Central metine bridge is reduced to **bilirubin**, a reddish-yellow pigment
3. Conjugation of bilirubin with glucuronic acid form bilirubin-diglucuronides, which are much more soluble than unconjugated bilirubin

- conjugated forms of bilirubin are secreted into the bile by an active transport mechanism, which is the rate-limiting step in bilirubin metabolism in the liver
Heme → heme oxygenase

heme oxygenase → CO → Fe^{2+}

Biliverdin

Biliverdin reductase

Biliverdin reductase → NADPH, H^+ → NADP^+

Bilirubin (in blood) → glucuronyl-bilirubin transferase → Bilirubin diglucuronide (liver)

Bilirubin diglucuronide transport to intestine → Bilirubin (in bile)

Bilirubin (in bile) → glucuronyl-bilirubin transferase → Bilirubin (in blood)

Bilirubin (in blood) as complex with serum albumin

Bilirubin (in blood) transport in blood

Bilirubin (in blood) transport to intestine

Bilirubin (in blood) transport to kidney

Urobinogen transport to intestine

Urobinogen transport to kidney

Urobinogen → Urobininogen

Urobininogen → Urobin

Urobininogen → Stercobilin
4. In the intestine, the glucuronic acid groups are removed from bilirubin by bacterial enzymes, and the bile pigment is reduced to urobilinogen, a colorless compound, which is oxidized to brown stercobilin and excreted in the feces

Abnormalities in heme metabolism

1. porphyrias – which result from defects in heme synthesis and

2. jaundice - which results from increased bilirubin levels in the blood
Biosynthese of AAs

oxaloacetate → aspartate

pyruvate → Ala, Val*, Leu*

α-ketoglutarate → glutamate

3-phosphoglycerate → serine

PEP + erythrose-4-P → phenylalanine*, Tyr, Trp*

Ribose-5-P → His*
Biosynthesis of AAs

Glycolytic, citric acid, and pentose phosphate intermediates

Gln and Glu are N sources

20 common amino acid pathways (bacterial)