Biosynthesis

Key Aspects

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Dec 2014
Format & Scope of Lecture

• **Enzyme cofactor chemistry:**
  – ATP, CoASH, SAM, DMAPP, biotin, NAD(P)+, NAD(P)H, FAD, FADH₂, peroxo-FADH₂, P₄₅₀ & PLP

• **Shikimate pathway:**
  – PEP + E-4-P → aromatic α-amino acids

• **Alkaloids**
  – Lys & Orn pathways to pyrrolidine & piperidine alkaloids – PLP chemistry
  – Phenolic coupling

• **Fatty acids and polyketides:**
  – The fatty acid synthase (FAS) iterative cycle
  – The polyketide synthase (PKS) iterative cycle(s)

• **Isoprenoids:**
  – The mevalonate pathway: 3× acetyl CoA → IPP & DMAPP
  – Pathways to linear C10, C15 & C20 isoprenoids: geranyl PP, farnesyl PP, geranylgeranyl PP
  – C30 isoprenoids via squalene synthase C15 head-to-head dimerisation
  – Basic carbocation chemistry: alkene cyclisation, elimination, trapping water, 1,2-alkyl & hydride shifts and modes of enzyme control thereof
**ATP - Free Energy Releasing Couple**

- **Key process:**

  \[
  \text{adenosine triphosphate (ATP)} + \text{ROH} \xrightarrow{\text{enzyme}} \text{ADP} + \text{P}_i
  \]

  \[\Delta G^\circ = -31 \text{ kJmol}^{-1}\]

- e.g. activation of bicarbonate in malonyl CoA biosynthesis (FA + PK lectures)

  \[
  \text{bicarbonate} \xrightarrow{\text{ATP}} \text{P}_i \xrightarrow{\text{biotin carboxylase}} \text{enolate of acetyl CoA} = \text{Nu}^\circ
  \]

  \[
  \text{bicarbonate} \xrightarrow{\text{ATP}} \text{P}_i \xrightarrow{\text{biotin carboxylase}} \text{transcarboxylase} \xrightarrow{\text{malonyl CoA}}
  \]
**CoASH - C-C Bond Formation**

- **Key processes:**

- e.g. iterative decarboxylative Claisen condensations in fatty acid biosynthesis (FA + PK lectures)

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**Diagram:**

- **Coenzyme A (CoASH):**
  - Pantetheinate
  - Coenzyme A (CoASH)

- **Adenosine:**

- **Enzyme:**
  - ACYL TRANSFER
  - α-CARBON ALKYLATION
  - ALDOL REACTIONS
  - CLAISEN-type C-ACYLATION

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**Diagram Description:**

- **Malonyl CoA**
  - **Decarboxylative Claisen condensation (dCoA):**
    - **2x NADPH**
    - **2x NADP**
    - **CO₂**
    - **Reduction [R]**
  - **Fatty acids**
**DMAPP - Dimethylallylation**

- **Key process:**
  - *NB. via allylic carbocation with trapping by nucleophile at either most or least substituted end...*

- *e.g. prenylation & reverse prenylation of alkaloids (alkaloid lectures)*

- **dimethylallyl pyrophosphate (DMAPP)**

- **lysergic acid** (halucinogen)

- **roquefortine** (blue cheese mould)

- **histidine**

- **tyrosine**
**SAM - Methylation**

### Key processes:

- S-adenosyl methionine (SAM)

- e.g. ketone C-methylation in citrinin biosynthesis (FA + PK lectures)

### Reaction Scheme:

1. **SAM Mediated Methylation**
   - **4x CoAS**
   - **5x CO₂**
   - **PKS**
   - **3x SAM**

2. **Citrinin Biosynthesis**
   - **Pentaketide**
   - **EnzS**
   - **Me**
   - **Me**
   - **CO₂**

### Enzyme Reactions:

- **enzyme**
  - **Nu-Me**
  - **Nu**
  - **Me**
  - **R₂N**
  - **Me-SR₂**
  - **Me-SR₂**
  - **Me-SR₂**

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**SAM** - S-adenosyl methionine; **PKS** - Polyketide Synthase; **SAM Mediated Methylation** - Methylation process facilitated by SAM; **Citrinin** - A type of mycotoxin.
**Biotin - Carboxylation**

- **Key process:**

  - *e.g.* carboxylation of acetyl CoA to give malonyl CoA (FA + PK lectures)

  ![Diagram of carboxylation reaction involving biotin and bicarbonate]

  - **bicarbonate**
  - **ATP** → **ADP**
  - **Nu** (bicarbonate)
  - **CoA**
  - **Mg<sup>2+</sup>**
  - **transcarboxylase**

  - **enolate of acetyl CoA = Nu<sup>−</sup>**
  - **malonyl CoA**

  - **biotin carboxylase**

  - **Nu = biotin**

  - **enolate of acetyl CoA**
NAD(P)⁺ - Oxidation

- Key process:
  - e.g. 1° alcohol to carboxylic acid oxidation in citrinin biosynthesis (FA + PK lectures)

Nicotinamide-adenine dinucleotide (NAD⁺) R = H

NB. NADP⁺: R = PO₃H⁻
Flavin - Oxidation

- Key process:
  - e.g. dehydrogenation of succinate $\rightarrow$ fumarate (citric acid cycle, alkaloid lectures)

- succinate $\rightarrow$ fumarate (Krebs cycle)
  - anti conformation imposed by enzyme via arginine salt bridges

\[
\begin{align*}
\text{succinate} & \rightarrow \text{fumarate} \\
\text{gauche} & \rightarrow \text{anti}
\end{align*}
\]
**Peroxyflavin - Oxidation**

- Key process:

  \[
  \text{O}_2 + \text{FADH}_2 \rightarrow \text{H}_2\text{O} + \text{FAD}
  \]

  - *e.g.* squalene $\rightarrow$ 2,3-oxidosqualene (isoprenoid lectures)

  - **squalene oxidase**

    \[
    \text{O}_2 + \text{FADH}_2 + \text{squalene} \rightarrow \text{2,3-oxidosqualene} + \text{H}_2\text{O} + \text{FAD}
    \]
$P_{450}$ Iron oxo - Oxidation

- **Key process:**

- **e.g. 1)** benzylic hydroxylation in citrinin biosynthesis (FA + PK lectures)
$P_{450}$ Iron oxo – Oxidation cont.

- *e.g.* 2) unactivated oxidative demethylation: $14\alpha$ demethylation in steroid biosynthesis (isoprenoid lectures)

- *e.g.* 3) activated oxidative demethylation: $4\alpha/\beta$ demethylation in steroid biosynthesis (isoprenoid lectures)
**P<sub>450</sub> Iron oxo** – Oxidation cont.

- *e.g. 4)* unactivated aromatic hydroxylation: phenylalanine → tyrosine in barley (alkaloid lectures)

  ![Diagram of unactivated aromatic hydroxylation]

- *e.g. 5)* activated aromatic hydroxylation: tyrosine → DOPA in opium (alkaloid lectures)

  ![Diagram of activated aromatic hydroxylation]
**NADPH - Reduction**

- **Key process:**
  
  - e.g. iminium ion to amine reduction in pyrrolizidine alkaloid biosynthesis (alkaloid lectures)

- **Formulae:**
  
  - Hydro-nicotinamide-adenine dinucleotide (NADPH) \( R = \text{PO}_3\text{H}^- \)
  
  - NB. NADH: \( R = \text{H} \)

- **Examples:**
  
  - NAD(P)H
  
  - 2x ornithine

- **Diagram:**
  
  - Spermidine \( \rightarrow \) NADH \( \rightarrow \) Homospermidine \( \rightarrow \) Retronecine

**Diagram Notes:**

- **NB.** \( H^2 = \text{H}^2 + 2\text{e} \)
PLP - Transamination

- Key process:

  e.g. oxidative deamination of cadaverine *en route* to piperidine alkaloids (alkaloid lectures)
**PLP - Decarboxylation**

- **Key process:**

  - e.g. lysine decarboxylation to cadaverine (alkaloid lectures)

- **Diagram:**

  - Enzyme-dependent decarboxylation process involving pyridoxal phosphate (PLP) as the coenzyme.
**PLP - Dealkylation**

- **Key process:**

  - e.g. serine side-chain cleavage → methanal (alkaloid lectures)

- **Diagram:**

- **PLP dependent dealkylation**

  - serine → methanal → N5-methyl-tetrahydrofolate → methionine → S-adenosyl methionine (SAM)
**Primary Metabolism - Overview**

**Primary metabolism**

1. **Photosynthesis**
   - CO₂ + H₂O → ATP and NADH
   - 'light reactions': hv → ATP and NADH
   - 'dark reactions': CO₂ → sugars (Calvin cycle)

2. **Glycolysis**
   - Glucose & other 4, 5, 6, & 7 carbon sugars

3. **Citric Acid Cycle**
   - Pyruvate → CO₂
   - Acetyl CoA → CO₂

**Primary metabolites**

- Oligosaccharides
- Polysaccharides
- Nucleic acids (RNA, DNA)

**Secondary metabolites**

- Shikimate metabolites
  - Cinnamic acid derivatives
  - Aromatic compounds
  - Lignans, flavonoids

- Alkaloids
  - Penicillins
  - Cephalosporins
  - Cyclic peptides

- Fatty acids & polyketides
  - Prostaglandins
  - Polycyclics
  - Aromatic compounds, polyphenols
  - Macrolides

- Isoprenoids
  - Terpenoids
  - Steroids
  - Carotenoids
The Shikimate Biosynthetic Pathway

- **Phosphoenol pyruvate & erythrose-4-phosphate → shikimate → chorismate → prephenate:**
Oxidative Phenolic Coupling

- **e.g. Morphine biosynthesis:** \( \sigma-/p\)-oxidative phenolic coupling:

- **e.g. Erysodine biosynthesis:** \( p-/p\)-oxidative phenolic coupling & dienone-phenol rearrangement:
Biosynthesis of Fatty Acids

- **Iterative oligomerisation via:**
  - Decarboxylative Claisen condensation
  - 3-step ketone reduction

```
CoAS
O
EnzS
O
SEnz
O
CO2
O
EnzS
EnzS
O O
EnzS
Malonyl CoA

2x NADPH
2x NADP+
+ H2O

Fatty acids
```

- **decarboxylative Claisen condensation**
  - dCc#1

- **reduction**
  - [R]#1
  - dCc#2

- **[R]#3**
- **dCc#3**
Introduction of Unsaturation

**ANAEROBIC ROUTE (bacteria)**
(dehydrogenation occurs during chain elongation)
mainly MUFAs but some PUFAs

**AEROBIC ROUTE (mammals, insects & plants)**
(dehydrogenation occurs after chain elongation)
MUFAs & PUFAs

**NB. in both cases cis-alkenes are produced**

vaccenic acid

oleic acid

PUFAs

plants

animals

C<sub>11</sub>-C<sub>12</sub>
Biosynthesis of Polyketides

- **Iterative oligomerisation via:**
  - Decarboxylative Claisen condensation
  - Variable levels of reductive ketone processing in each iteration

![Chemical reaction diagram involving CoA, enzymes, and polyketides](image)

- **Example:**
  - $R' = H$
  - $R' = Me$
  - $R' = Et$

- **Decarboxylative Claisen condensation** $\text{dCc}^1$

- **Linear & cyclised polyketides**

- **[funct]$^3$**
  - $\text{EnzS} \rightarrow \text{EnzS}$
  - $\text{EnzS}$

- **[funct]$^2$**
  - $\text{EnzS}$
  - $\text{EnzS}$

- **[funct]$^1$**
  - $\text{EnzS}$
  - $\text{EnzS}$

- **no KR**
- **no DH**
- **no ER**

- **$dCc^1$**
- **$dCc^2$**
- **$dCc^3$**
Biosynthesis of IPP & DMAPP

- acetyl CoA → acetoacetyl CoA → HMG CoA → mevalonate → IPP → DMAPP:

\[\begin{align*}
&\text{acetyl CoA} \\
\rightarrow &\text{acetoacetyl CoA} \\
\rightarrow &\text{HMG CoA} \\
\rightarrow &\text{mevalonate} \\
\rightarrow &\text{IPP} \\
\rightarrow &\text{DMAPP:}
\end{align*}\]
‘Head-to-tail’ Oligomerisation → Isoprenoids

DMAPP $\xrightarrow{S_n_1}$ intimate ion pair

DMAPP $\xrightarrow{S_n_1}$ IPP

IPP $\xrightarrow{S_n_1}$ geranyl pyrophosphate ($C_{10}$)

IPP $\xrightarrow{S_n_1}$ farnesyl pyrophosphate ($C_{15}$)

IPP $\xrightarrow{S_n_1}$ geranylgeranyl pyrophosphate ($C_{20}$)

DMAPP $\xrightarrow{S_n_1}$ MONOTERPENES ($C_{10}$)

IPP $\xrightarrow{S_n_1}$ SESQUITERPENES ($C_{15}$)

IPP $\xrightarrow{S_n_1}$ TRITERPENES ($C_{30}$)

IPP $\xrightarrow{S_n_1}$ DITERPENES ($C_{20}$)

IPP $\xrightarrow{S_n_1}$ CAROTENOIDs ($C_{40}$)
Triterpenes – $2 \times FPP \ 'head-to-head' \rightarrow Squalene$

EnzB:

FPP (donor) → presqualene PP

Squalene synthase:

Presqualene PP → Squalene

NADPH + $\text{PP}_i$ → NAD + $\text{P}_i$

Squalene → Triterpene derivatives e.g. steroids
Terpene Cyclases – Control of Cyclisation

- **Functional aspects of terpenoid cyclases:**
  - **Templating:** Active site provides a template for a specific conformation of the flexible linear isoprenoid starting material.
  - **Triggering:** Cyclase initiates carbocation formation.
    - Metal-assisted leaving group departure (e.g. pyrophosphate ionization aided by Mg^{2+})
    - C=C bond protonation (e.g. squalene-hopene cyclase, see later).
    - Epoxide protonation (e.g. oxidosqualene cyclase, see later).
  - **Chaperoning:** Chaperones conformations of carbocationic intermediates through the reaction sequence, ordinarily leading to one specific product.
  - **Sequestering:** Sequesters the carbocation intermediates by burying the substrate in a hydrophobic cavity that is generally solvent-inaccessible. Carbocations are concomitantly stabilized by the presence of aromatic residues in the active site that exert their effects via cation-\pi interactions.
Primary Metabolism - Overview

Primary metabolism

\[ \text{CO}_2 + \text{H}_2\text{O} \]

1) ‘light reactions’: \(\text{hv} \rightarrow \text{ATP and NADH}\)
2) ‘dark reactions’: \(\text{CO}_2 \rightarrow \text{sugars (Calvin cycle)}\)

<table>
<thead>
<tr>
<th>Glycolysis</th>
<th>Glucose &amp; other 4,5,6 &amp; 7 carbon sugars</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{PHOTOSYNTHESIS})</td>
<td>(\text{hv} \rightarrow \text{ATP and NADH})</td>
</tr>
<tr>
<td>(\text{CO}_2 \rightarrow \text{sugars (Calvin cycle)})</td>
<td>(\text{glycolysis})</td>
</tr>
<tr>
<td>(\text{phosphoenol pyruvate} \rightarrow \text{pyruvate})</td>
<td>(\text{emanose-4-phosphate} \rightarrow \text{shikimate})</td>
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<tr>
<td>(\text{citric acid cycle (Krebs cycle)})</td>
<td>(\text{aromatic amino acids} \rightarrow \text{peptides})</td>
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<tr>
<td>(\text{acetyl coenzyme A} \rightarrow \text{malonyl coenzyme A})</td>
<td>(\text{allphatic amino acids} \rightarrow \text{proteins})</td>
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<tr>
<td>(\text{mevalonate})</td>
<td>(\text{saturated fatty acids} \rightarrow \text{lipids})</td>
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<td>(\text{phosphoenol pyruvate})</td>
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<td>(\text{CO}_2 + \text{H}_2\text{O})</td>
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<td>(\text{carotenoids})</td>
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Acknowledgements

• I would like to acknowledge the following for kindly allowing me to consult and use material from their lecture courses on various aspects of biosynthesis:

  - Dr Finian Leeper, Dept. of Chemistry, University of Cambridge  
    http://www-leeper.ch.cam.ac.uk

  - Dr John McKendrick, Dept. of Chemistry, University of Reading  
    http://www.chem.rdg.ac.uk/dept/staff/org/jem.html

  - Dr David Widdowson, Dept. of Chemistry, Imperial College London  
    http://www.ch.ic.ac.uk/widdowson/

• Additionally, I have adapted ideas from several web-sites & in particular I have adapted material from two biological chemistry courses at Harvard University & MIT:
  - http://www.courses.fas.harvard.edu/%7echem27/
  - http://ocw.mit.edu/OcwWeb/Chemistry/5-08JSpring2004/CourseHome/index.htm

• Other reference sources have been the books cited in the ‘course overview’, particularly:
  - J. Mann, ‘Chemical Aspects of Biosynthesis’, Oxford Chemistry Primer No. 20, 1994
  - J. Mann, ‘Secondary Metabolism’, Oxford University Press, 2nd ed. 1987