Biochemistry 2

SS2020
Office hours

- Office: A F1.7
- Office hours: Tue 8:00-11:00 and Thr 8:00-10:00
- ahromic@ius.edu.ba
Grading

- Final exam 40%
- Interm 60%:
  - Lab tutorials (working in the lab and lab reports) 20%
  - Quizes (2 in total) 15%
  - Midterm exam (everything from the beginning of semester) 25%
Tutorials

- 6 in total
- Assistant: MSc Muhamed Adilovic
Teaching methods

- Slides
- Books:
  - b-ok.org
Schedule

- Tue 11:00-13:00
- Thr 10:00-11:00
<table>
<thead>
<tr>
<th>WEEK</th>
<th>TOPIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>Introduction and syllabus</td>
</tr>
<tr>
<td>Week 2</td>
<td>Introduction to Biochemistry/The beginning of biochemistry</td>
</tr>
<tr>
<td>Week 3</td>
<td>Amino acids and the primary structure of the proteins</td>
</tr>
<tr>
<td>Week 4</td>
<td>Complexity of proteins and structure determination</td>
</tr>
<tr>
<td>Week 5</td>
<td>Enzyme catalysis</td>
</tr>
<tr>
<td>Week 6</td>
<td>Enzyme classification</td>
</tr>
<tr>
<td>Week 7</td>
<td>Overview on carbohydrates and lipids</td>
</tr>
<tr>
<td>Week 8</td>
<td><strong>MIDTERM WEEK</strong></td>
</tr>
<tr>
<td>Week 9</td>
<td>Biological membranes and transport</td>
</tr>
<tr>
<td>Week 10</td>
<td>Cofactors and coenzymes</td>
</tr>
<tr>
<td>Week 11</td>
<td>Overview of metabolism/Glycolysis</td>
</tr>
<tr>
<td>Week 12</td>
<td>Citric acid cycle/Electron transport</td>
</tr>
<tr>
<td>Week 13</td>
<td>Hormones</td>
</tr>
<tr>
<td>Week 14</td>
<td>Hormonal Regulation of Mammalian Metabolism</td>
</tr>
<tr>
<td>Week 15</td>
<td>Review and final exam preparations</td>
</tr>
<tr>
<td>WEEK</td>
<td>TOPIC</td>
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</tr>
<tr>
<td>Week 1</td>
<td>Introduction and syllabus</td>
</tr>
<tr>
<td>Week 2</td>
<td>Biosynthesis of fatty acids, terpenes and steroids</td>
</tr>
<tr>
<td>Week 3</td>
<td>Biosynthesis of amino acids</td>
</tr>
<tr>
<td>Week 4</td>
<td>Biosynthesis using amino acids: Heme, active amines and alkaloids</td>
</tr>
<tr>
<td>Week 5</td>
<td>Biosynthesis of ribonucleotides and deoxyribonucleotides</td>
</tr>
<tr>
<td>Week 6</td>
<td>DNA replication, repair and recombination</td>
</tr>
<tr>
<td>Week 7</td>
<td>MIDTERM</td>
</tr>
<tr>
<td>Week 8</td>
<td>Transcription</td>
</tr>
<tr>
<td>Week 9</td>
<td>mRNA processing</td>
</tr>
<tr>
<td>Week 10</td>
<td>Translation and protein synthesis</td>
</tr>
<tr>
<td>Week 11</td>
<td>Protein characterization methods</td>
</tr>
<tr>
<td>Week 12</td>
<td>Biochemistry of plants: Photosynthesis</td>
</tr>
<tr>
<td>Week 13</td>
<td>Dark reactions: Calvin cycle</td>
</tr>
<tr>
<td>Week 14</td>
<td>The C4 pathway</td>
</tr>
<tr>
<td>Week 15</td>
<td>Review and final exam preparations</td>
</tr>
</tbody>
</table>
Biosynthesis of fatty acids, terpenes and steroids
Beta oxidation

• Catabolic process
• Fatty acids broken to generate acetyl-CoA
• Enters citric acid cycle, NADH and FADH2
• Coenzymes of electron transport chain
• Name: beta carbon of the fatty acid undergoes oxidation to a carbonyl group
• Facilitated by mitochondrial trifunctional protein – enzyme complex
Fatty acid biosynthesis

- Reverse of beta oxidation
- Achieved by condensation of C2 units
- Donor of units: malonyl-CoA
The tricarboxylate transport system

- FA synthesis – cytoplasm
- Starting material – malonyl-CoA
- Largerly formed in mitochondrion by FA oxidation
- Impermeable for acetyl-CoA
- Special transport system – shuttles acetyl-CoA to the cytosol
- Via citrate
Acetyl-CoA carboxylase

- first step in fatty acid biosynthesis - biotin-dependent carboxylation of acetyl-CoA to malonyl-CoA

- Acetyl-CoA carboxylase – 2 types of regulation:
  1. allosteric (citrate activates, long-chain fatty acids inhibit)
  2. hormonal mechanisms (insulin)
Acetyl-CoA carboxylase

- several phosphorylation sites
- affects the activity of the enzyme directly
- Note: prokaryotes synthesize fatty acids as precursors for phospholipids (membrane)
- not to make triacylglycerols for energy storage
The acyl carrier protein (ACP)

- fatty acid synthesis starts with two CoA derivatives
- acyl group is transferred to an acyl-carrier protein (ACP)
- tethers the acyl group by the same phosphopantetheine prosthetic group to a serine
- In bacteria ACP is a small 10 kDa protein
- In animals it is part of a multifunctional fatty acid synthase complex
Primming reactions: “Loading fatty acid synthase”

- Fatty acid synthase is loaded with the acyl precursors
- Malonyl-CoA and acetyl-CoA are transferred to ACP (1 and 2b)
- Acetyl-ACP is further transferred to an enzyme cysteine group (2a)
- Condensation reaction then generates acetoacetyl-CoA
- Enzymes:
  1. Acetyl-CoA-ACP transacylase
  2. β-ketoacyl-ACP synthase
  3. Malonyl-CoA-ACP transacylase

Chapter 1
Mechanism of the condensing reaction

- decarboxylation of malonyl-CoA - leads to a carbanion
- Nucleophilically attacks the carbonyl group of acetyl-CoA
- drives the endergonic C-C bond formation
- energy „contained“ in malonyl-CoA derives from the previous ATP consuming acetyl-CoA carboxylation reaction
- similar situation is found in gluconeogenesis
Reduction & dehydration

- Acetoacetyl-ACP reduced to butyryl-ACP
- Two reduction and a dehydration step
- butyryl-ACP - transferred to the cysteine group of the enzyme
- for the next round of reactions
- Enzymes:
  4. $\beta$-ketoacyl-ACP reductase
  5. $\beta$-hydroxyacyl-ACP dehydrase
  6. enoyl-ACP reductase
Stoichiometry of fatty acid synthesis

- Example: synthesis of C-16 fatty acid (palmitoily-ACP)
- Fatty acid synthase has to add seven malonyl-ACP to the growing fatty acid

\[
\text{acetyl-CoA} + 7 \text{malonyl-CoA} + 14 \text{NADPH} + 14 \text{H}^+ \\
\text{palmitate} + 7 \text{CO}_2 + 14 \text{NADP}^+ + 8 \text{CoA} + 6 \text{H}_2\text{O}
\]

- Malonyl-CoA is derived from acetyl-CoA

\[
7 \text{acetyl-CoA} + 7 \text{CO}_2 + 7 \text{ATP} \rightarrow 7 \text{malonyl-CoA} + 7 \text{ADP} + 7 \text{Pi} + 7 \text{H}^+
\]

Overall reaction:

\[
8 \text{acetyl-CoA} + 7 \text{ATP} + 14 \text{NADPH} + 7 \text{H}^+ \\
\text{palmitate} + 7 \text{ADP} + 7 \text{Pi} + 14 \text{NADP}^+ + 6 \text{H}_2\text{O} + 8 \text{CoA}
\]
Elongases and desaturases

- Palmitate - end product of fatty acid synthase (in animals)
- Converted to longer fatty acids by elongases
- Double bonds introduced by desaturases
- Enzymes - present in mitochondria and the endoplasmic reticulum
Elongases

- Elongases in mitochondria - extend fatty acids
- process essentially the reverse of β-oxidation
- elongases in the ER - operate via condensation of malonyl-CoA to acyl-CoAs
Desaturases

- NAD+-dependent non-heme iron proteins
- exist in mammals with specific activity
- not all fatty acid can be synthesized by mammals
- *linoleic acid* - only be made by plants
- they have the required desaturases
- these fatty acid needs to be taken up with the diet
- *essential fatty acid*
Regulation of fatty acid metabolism

- insulin has the opposite effect on fatty acid metabolism!
- Glucagon/epinephrine
- Increases cAMP in adipose cells
- Aktivates cAPK
- inactivates acetyl-CoA carboxylase, i.e. fatty acid biosynthesis
Chapter 1

Summary of lipid metabolism

- Triacylglycerols
- Fatty acids
- Membrane lipids
- Ketone bodies
- Acetyl-CoA
- Cholesterol/Terpenes

\[ \beta \text{-oxidation} \]
\[ \text{fatty acid biosynthesis} \]

\[ \text{FADH}_2/\text{NADH} \]
\[ \text{NADPH/ATP} \]

\[ \text{citric acid cycle} \]

\[ \text{oxidative phosphorylation} \]

\[ \text{ATP} \]
\[ \text{FADH}_2/\text{NADH/GTP} \]
Summary

- Difference between beta oxidation and fatty acid synthesis
- Transport system
- Acetyl-CoA carboxylase
- ACP
- Priming reactions
- Condensation, reduction and dehydration
- Elongases and desaturases
- Regulation of FA metabolism
Terpenes & sterols – the isoprenoids

- Acetyl-SCoA - building block of a variety of hydrophobic compounds collectively called isoprenoids
- Large family of natural products with diverse structures
- Major classes

- Terpenes
  - Monoterpenes (C10)
  - Sesquiterpenes (C15)
  - Diterpenes (C20)
  - Sesterterpenes (C25)
  - Triterpenes (C30) etc.

- Sterols
  - Triterpenes (C30)

- Carotenoids
  - Tetraterpenes (C40)
Terpenes

- More than 20000 terpenes are known
- Some terpenes are hydrocarbons
- Other contain oxygen and are alcohols, aldehydes or ketones
- Oxygen containing terpenes - called terpenoids
- Have been used as spices, perfumes and medicines

- Menthol (peppermint oil)
- Geraniol (geranium oil)
- Zingiberene (oil of ginger)
- β-Selinene (oil of celery)
Examples

• Absinthe
• Extracts of the plant *Artemisia absinthium*
• used to prepare the green alcoholic beverage absinthe
• main compound is *thujone*
• psychoactive compound with severe long term neurotoxic effects
• extract contains *absinthine* – most bitter substances known

Chapter 1

[Chemical structures of thujone and absinthine]
Absinthe - Rise and fall

- first produced around 1792 in western Switzerland
- preferred alcoholic beverage of the bohemian society in France in the 19th century
- particularly fashionable among writers, poets and artists
- 1850ties the adverse effects – seizures, hallucinations
- total ban in most countries in the early 20th century
Artemisinin - a sesquiterpene lactone

- *Artemisia annua* (sweet wormwood)
- produces a sesquiterpene lactone with an unusual peroxide bridge
Artemisinin has antimalarial properties

- China & Vietnam - used a traditional medicine against fever
- plant requires special climatological and agricultural condition in order to produce artemisinin
- kills the malaria parasites during this developmental stage
Rubber - a natural isoprene biopolymer

- *Hevea brasiliensis* – white liquid (when cutting the bark of a tree)
- sticky liquid (latex) – collected and used for the production of rubber
- Natural rubber - polymer of isoprene, containing an average of 5000 isoprene units

\[
\text{isoprene} \quad \xrightarrow{n} \quad Cis\text{-poly}(2\text{-methyl}-1,3\text{-butadiene})
\]

\[
\text{Natural rubber}
\]
Biosynthesis of terpenes

- does not use isoprene
- similar carbon skeleton in the form of *isopentenyl pyrophosphate* is used as a building block
- starts with acetyl-CoA
Formation of isopentenyl pyrophosphate from HMG-CoA

1: HMG-CoA reductase
2: mevalonate-5-phosphotransferase
3: phosphomevalonate kinase
4: pyrophosphomevalonate decarboxylase
Pyrophosphomevalonate decarboxylase

- ATP-dependent decarboxylation - last step in isopentenyl pyrophosphate synthesis
- Decarboxylation and dehydration/elimination occur simultaneously - concerted fashion
HMG-CoA reductase

- catalyzes the rate-determining step in the biosynthesis of terpenes and steroids
- most elaborately regulated enzyme of the pathway
- particular importance for cholesterol biosynthesis
- HMG-CoA reductase – prominent target for inhibitors in order to reduce blood cholesterol levels
- Statins - derivatives of the fungal products compactin and lovastatin
- competitive inhibitors of the enzyme
Isopentenyl pyrophosphate isomerase

- Biosynthesis of terpenes, sterols and carotenoids requires a second substrate
- formed by isomerization of the isopentenylpyrophosphate
- involve concerted acid-base catalysis
Prenyl transferase

- catalyzes two head-to-tail condensations:
  - 1. joins dimethylallylpyrophosphate and isopentenyl pyrophosphate to geranyl pyrophosphate
  - 2. geranyl pyrophosphate and isopentenyl pyrophosphate to farnesyl pyrophosphate (a C15 compound)
Squalene synthase

- catalyzes the head-to-head condensation of two farnesyl pyrophosphate molecules to give squalene
Lanosterol is synthesized from squalene

- open chain compound squalene is cyclized
- to the first tetracyclic compound, lanosterol
- Lanosterol is then transformed to cholesterol
- a sequence of 19 steps (!) to yield cholesterol
Cholesterol utilization

- used to synthesize **bile acids** and **steroid hormones**
- Five classes:
  - progestins
  - glucocorticoids
  - mineralcorticoids
  - androgens
  - estrogens
- variety of different physiological processes
Formation of bile acids (salts)

- synthesized in the liver
- glycine or taurine conjugates and secreted into the gallbladder
- secreted into the small intestine
- act as emulsifiers in the digestion of fats
Summary of terpene and sterol biosynthesis

Geranyl pyrophosphate

+IPP

Farnesyl pyrophosphate

+IPP

Squalene

+IPP

Geranylgeranyl pyrophosphate

monoterpane cyclases

Monoterpenes

Sesquiterpenes

Triterpenes & Steroids

Diterpenes

Chapter 1
IPP is a central metabolite of many biosynthetic pathways
Summary

- Classes of terpens and sterols
- Examples and applications
- Biosynthesis of terpens
- Squalene synthase
- Cholesterol utilization
- Function of IPP