Biochemistry

Metabolic pathways

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Objectives of the course:

**Energy metabolism**

- high-energy donors
- coupled reactions
- co-factors: NAD⁺/NADH; FAD/FADH₂; TPP; PLP; CoA; Biotin
- glycolysis/glyconeogenesis
- citric acid cycle: regulation (tissue-dependent)
  - GABA-shunt;
  - glyoxylate cycle
- respiratory chain and oxidative phosphorylation
- glycogen metabolism (activated glucose)
- pentose phosphate pathway (tissue specificity)
- photosynthesis: RUBISCO
  - C4-plants
Nitrogen metabolism

$N_2$ assimilation via reduction to NH$_3$ (nitrogenase complex)
NH$_3$ metabolism: glutamate-dehydrogenase
  glutamate synthase
  glutamine synthetase
  glutamine amidotransferase
utra cycle
C$_1$ metabolism (PLP, THF, SAM, homocystein)
nucleotide metabolism: biosynthesis of purines and pyrimidines
  from RNA to DNA (NDP reductase)
  salvage pathway, HGPRT deficiency
cytostatic drugs
catabolism (ADA-deficiency, urate)

Signal transduction

$G$PCR - glucagon signalling
RTK - insulin signalling
$G$-proteins
Structural hierarchy in the molecular organization of cells

Lehninger Principles of Biochemistry. 5th Ed.
Nelson & Cox
Essential Questions?

- What are the properties of regulatory enzymes?
- How do regulatory enzymes sense the momentary needs of cells?
- What molecular mechanisms are used to regulate enzyme activity?
- Factors that influence enzymatic activity
- General features of allosteric regulation
- The kind of covalent modification that regulates the activity of enzymes
- Is the activity of some enzymes controlled by both allosteric regulation and covalent modification?
- Special focus: is there an example in nature that exemplifies the relationship between quaternary structure and the emergence of allosteric properties? hemoglobin and myoglobin – paradigms of protein structure and function
What Factors Influence Enzymatic Activity?

- The **availability of substrates and cofactors** usually determines how fast the reaction goes.
- **K<sub>m</sub>** values ~ the prevailing *in vivo* concentration of the substrates.
- As product accumulates, the apparent rate of the enzymatic reaction will decrease as equilibrium is approached.

- **Genetic regulation of enzyme synthesis and decay** determines the amount of enzyme present at any moment.
  - **Induction** = activation of enzyme synthesis
  - **Repression** = shutdown of enzyme synthesis
  - By controlling the [E], cells can activate or terminate metabolic routes.
  - Other factors: **Zymogens, isozymes, and modulator** proteins may play a role.
Principal characteristics of metabolic pathways

1. Metabolic pathways are irreversible.
2. Catabolic and anabolic pathways must differ.
3. Every metabolic pathway has a first committed step.
4. All metabolic pathways are regulated.
5. M.p. in eukaryotic cells occur in specific subcellular compartments.
Parallel pathways of catabolism and anabolism **must differ in at least one metabolic step** in order that they can be regulated independently. Shown here are two possible arrangements of opposing catabolic and anabolic sequences between A and P. (a) Parallel sequences proceed by independent routes. (b) Only one reaction has two different enzymes.
Metabolic Pathways are Compartmentalized within Cells

• Eukaryotic cells are compartmentalized by an endomembrane system - advantageous for metabolism
• Each organelle (compartment) - dedicated to specialized metabolic functions and contains appropriate enzymes - confined together.

• Advantages:
  – Allow analyses of respective functions because they can be separated
  – Enzymes are isolated from competing pathways
  – Temporal compartmentalization
  – Intermediates are spatially and chemically segregated
  – Genes of metabolism show a circadian pattern of regulated expression
  – Example: glucose-6 phosphatase that converts glucose-6-phosphate to glucose is localized in the ER.
Where metabolic processes occur at the organ level

Liver
- Liver is the center of metabolism - maintains blood glucose levels and regulates the concentration of metabolites in the blood.
- Stores glycogen that can be made into glucose-6-phosphate, then glucose.
- Makes glucose by gluconeogenesis (from pyruvate, de novo).
- Synthesizes FA, cholesterol and bile salts.
- Produces ketone bodies but cannot use them (no CoA transferase in the liver)
- Only the liver and kidneys contain glucose-6-phosphatase

Glucose regulation in the liver
- When blood glucose is high (fed state), FA are synthesized by the liver, converted into triacylglycerols and packaged into VLDL that are secreted into the blood.
- When blood glucose is low (fasting state), the liver produces ketone bodies to fuel the heart and muscle to preserve glucose for the brain. Eventually brain is fed by ketone bodies.
Other organs in metabolism

- **Brain** - Glucose is the primary fuel; only after prolonged fasting (not eating) does the brain use ketone bodies for fuel (last resort).
  - Brain has no capacity to store fuel - needs a constant supply
  - Consumes a lot of energy - 120 g of glucose per day.
  - Glucose is transported into the brain by GLU3 glucose transporter (crosses the membrane).
  - [glucose] in brain - maintained around 5 mM so glucose is saturated under normal conditions. If drops to 2.2 mM the brain is in trouble.
- **Muscle** - uses glucose, FA and ketone bodies for fuel; have stores of glycogen that is converted to glucose when needed for bursts of activity.
- **Intestines**
- **Kidney**
- **Adipose tissue**
- **Heart**
Metabolic principle: Degradation is coupled to the formation of ATP (energy store) and NADPH (reduction equivalents), that represent sources of free energy for biosynthetic reactions.
Overview of catabolism

high-enthalpy, low-entropy

low-enthalpy, high-entropy

Biochemistry. Voet & Voet
Stage 1: Proteins, polysaccharides and lipids are broken down into their component building blocks.

Stage 2: The building blocks are degraded into the common product, generally the acetyl groups of acetyl-CoA.

Stage 3: Catabolism converges to three principal end products: water, carbon dioxide, and ammonia.
The central role of ATP for energy exchange in biological systems was discovered in 1941 by Fritz Lipmann und Herman Kalckar.

ATP-turnover: 40 kg/day

Energy rich compounds: the triphosphate subunit, contains 2 phosphoric acid anhydride linkages, which upon hydrolysis release high amounts of energy:

\[
\begin{align*}
\text{ATP} + \text{H}_2\text{O} & \leftrightarrow \text{ADP} + \text{Pi} + \text{H}^+ \quad \Delta G^\circ = -30,5 \text{ kJ/mol} \\
\text{ATP} + \text{H}_2\text{O} & \leftrightarrow \text{AMP} + \text{PPi} + \text{H}^+ \quad \Delta G^\circ = -30,5 \text{ kJ/mol} \\
\end{align*}
\]

ATP, ADP, AMP, interconversionable: adenylate-kinase:

\[
\begin{align*}
\text{ATP} + \text{AMP} & \leftrightarrow \text{ADP} + \text{ADP}
\end{align*}
\]

ATP is continuously formed and hydrolysed.
Transfer of the phosphate group from "high-energy" donors via ATP to "low-energy" acceptors:

![Diagram showing energy levels of phosphate compounds]

- Phosphoenolpyruvate
- 1,3-Bisphosphoglycerate
- Phosphocreatine
- ATP
- Glucose-6-phosphate
- Glycerol-3-phosphate

"High-energy" phosphate compounds

"Low-energy" phosphate compounds

Biochemistry. Voet & Voet
**Lehninger Principles of Biochemistry. 5th Ed.**

**Diagram:**

1. **ATP**\(^{4-}\) + \(H_2O\) → **ADP**\(^{3-}\) + \(P_i^{2-}\) + \(H^+\)  
\[\Delta G^\circ = -30.5 \text{ kJ/mol}\]  

2. **PEP**\(^{3-}\) + \(H_2O\) → pyruvate\(^{-}\) + \(P_i^{2-}\)  
\[\Delta G^\circ = -61.9 \text{ kJ/mol}\]  

3. **Phosphocreatine**\(^{2-}\) + \(H_2O\) → creatine + \(P_i^{2-}\)  
\[\Delta G^\circ = -43.0 \text{ kJ/mol}\]
When a sudden demand of energy depletes ATP, the PCr reservoir is used to replenish ATP at a rate considerably faster than possible via catabolism. Vice versa, when demand of energy is reduced, ATP produced by catabolism is used to replenish the PCr reservoir.
Structure of Acetyl-CoA:

a „high-energy“ thioester of the nucleotide cofactor Coenzyme A

\[ \Delta G^0' = -31 \text{ kJ/mol} \]
Acetyl-CoA + H₂O → acetate⁻ + CoA + H⁺

ΔG° = -31.4 kJ/mol
Lehninger Principles of Biochemistry. 5th Ed.
Nelson & Cox
The central role of Acetyl-CoA in metabolism:

**Citric acid cycle**

**Metabolic intermediate:**
- Fatty acid metabolism
- Carbohydrate metabolism
- Amino acid metabolism
- Precursor of cholesterol and steroid hormones

**Acetyl-group donor:**
- Choline: Acetylcholine (neurotransmitter)
- Lys of histones

*Biochemistry. Voet & Voet*
Three types of nonlinear metabolic pathways

(a) Converging catabolism

(b) Diverging anabolism

(c) Cyclic pathway

Lehninger Principles of Biochemistry. 5th Ed.
Nelson & Cox
NAD$^+$ and NADP$^+$ accept e$^-$ only pairwise ("parking" an hydride ion)
<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Coenzyme</th>
<th>Stereochemical specificity for nicotinamide ring (A or B)</th>
<th>Text page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isocitrate dehydrogenase</td>
<td>NAD⁺</td>
<td>A</td>
<td>610</td>
</tr>
<tr>
<td>α-Ketoglutarate dehydrogenase</td>
<td>NAD⁺</td>
<td>B</td>
<td>610</td>
</tr>
<tr>
<td>Glucose 6-phosphate dehydrogenase</td>
<td>NADP⁺</td>
<td>B</td>
<td>540</td>
</tr>
<tr>
<td>Malate dehydrogenase</td>
<td>NAD⁺</td>
<td>A</td>
<td>612</td>
</tr>
<tr>
<td>Glutamate dehydrogenase</td>
<td>NAD⁺ or NADP⁺</td>
<td>B</td>
<td>665</td>
</tr>
<tr>
<td>Glyceraldehyde 3-phosphate dehydrogenase</td>
<td>NAD⁺</td>
<td>B</td>
<td>530</td>
</tr>
<tr>
<td>Lactate dehydrogenase</td>
<td>NAD⁺</td>
<td>A</td>
<td>538</td>
</tr>
<tr>
<td>Alcohol dehydrogenase</td>
<td>NAD⁺</td>
<td>A</td>
<td>540</td>
</tr>
</tbody>
</table>
Enzymes: Oxidoreductases/Dehydrogenases

NAD$^+$ and NADP$^+$, NADH and NADPH are cofactors in more than 200 reactions (type electron transfer):

$$\text{AH}_2 + \text{NAD(P)}^+ \leftrightarrow \text{A} + \text{NAD(P)H} + \text{H}^+$$

Alcohol-dehydrogenase

$$\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ \leftrightarrow \text{CH}_3\text{CHO} + \text{NADH} + \text{H}^+$$
<table>
<thead>
<tr>
<th>Reaction</th>
<th>Concentration</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[\text{NAD}^+] \gg [\text{NADH}]$</td>
<td>$\approx [10^{-5} \text{ M}]$</td>
<td>Enables catabolic oxidations</td>
</tr>
<tr>
<td>$[\text{NADP}^+] \ll [\text{NADPH}]$</td>
<td>$\approx [10^{-6} \text{ M}]$</td>
<td>Enables anabolic reductions</td>
</tr>
</tbody>
</table>

Niacin deficiency causes Pellagra (3 Ds: Dermatitis, Diarrhoea, Dementia).
An inability to absorb niacin (vitamin B3) or the amino acid tryptophan may cause pellagra, a disease characterized by scaly sores, mucosal changes and mental symptoms.
FMN and FAD are able to sequentially transfer one e⁻
# TABLE 13–9  Some Enzymes (Flavoproteins) That Employ Flavin Nucleotide Coenzymes

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Flavin nucleotide</th>
<th>Text page(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acyl-CoA dehydrogenase</td>
<td>FAD</td>
<td>638</td>
</tr>
<tr>
<td>Dihydrolipoyl dehydrogenase</td>
<td>FAD</td>
<td>605</td>
</tr>
<tr>
<td>Succinate dehydrogenase</td>
<td>FAD</td>
<td>612</td>
</tr>
<tr>
<td>Glycerol 3-phosphate dehydrogenase</td>
<td>FAD</td>
<td>714–715</td>
</tr>
<tr>
<td>Thioredoxin reductase</td>
<td>FAD</td>
<td>869</td>
</tr>
<tr>
<td>NADH dehydrogenase (Complex I)</td>
<td>FMN</td>
<td>696–697</td>
</tr>
<tr>
<td>Glycolate oxidase</td>
<td>FMN</td>
<td>767</td>
</tr>
</tbody>
</table>
Non-enzymatic glucosylation of proteins

Reversible generation of a Schiff base

Irreversible Amadori-rearrangement