BIO506 – BIOCHEMISTRY II

MID TERM IMPORTANT QUESTIONS

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1. What are competitive and non-competitive inhibitors?

Competitive inhibitors

- Inhibitors that resemble the substrate and compete with it for binding at the active site of the enzyme.
- Their effect can be reduced or overcome by increasing substrate concentration.
- They increase the apparent Km (decrease affinity) but do not change Vmax.

Non-competitive inhibitors

- Inhibitors that bind to a site other than the active site (allosteric site) on the enzyme.
- They do not compete with the substrate for the active site, so increasing substrate concentration does not overcome inhibition.
- They decrease Vmax but Km remains unchanged.

2. Compare ATP with other energy rich compounds.

Comparison of ATP with other energy-rich compounds

ATP

- Contains phosphoanhydride bonds.
- Standard free energy of hydrolysis ($\Delta G^{\circ\prime}$) ≈ −7.3 kcal/mol (−30.5 kJ/mol).
- Acts as the universal energy currency of the cell.
- Energy transfer mainly by phosphorylation.
- Intermediate energy-rich compound.

Other energy-rich compounds

- Include phosphoenolpyruvate (PEP), 1,3-bisphosphoglycerate, creatine phosphate, and acetyl-CoA.
- Possess enol phosphate, acyl phosphate, guanidino phosphate, or thioester bonds.
- Have higher free energy of hydrolysis than ATP (e.g., PEP ≈ −14.8 kcal/mol).
- Mainly act as energy reservoirs or intermediates in metabolic pathways.
- Can transfer phosphate or acyl groups to ADP to form ATP.

3. Write role of GTP in substrate level phosphorylation.

Role of GTP in substrate-level phosphorylation

- In the TCA cycle, during conversion of succinyl-CoA to succinate, energy from the thioester bond is directly used to phosphorylate GDP to GTP.
- This reaction is catalyzed by succinyl-CoA synthetase (succinate thiokinase).
- GTP formed represents substrate-level phosphorylation, as ATP/GTP is generated directly without involvement of the electron transport chain.
- GTP can readily transfer its phosphate to ADP to form ATP via nucleoside diphosphate kinase.
- In liver and kidney, GTP is preferentially used in energy-requiring processes such as gluconeogenesis.

4. Differentiate between ETC and uncoupler.

The electron transport chain (ETC) is a series of electron carriers located in the inner mitochondrial membrane that transfer electrons from NADH and $FADH_2$ to molecular oxygen. During this process, protons are pumped across the membrane, creating an electrochemical gradient that drives ATP synthesis through oxidative phosphorylation. In contrast, an uncoupler is a substance that disrupts the link between electron transport and ATP formation by allowing protons to flow back into the mitochondrial matrix without passing through ATP synthase. As a result, electron transport and oxygen consumption continue or even increase, but ATP synthesis is reduced or completely inhibited, and the released energy is dissipated as heat.

5. Write names of allosteric activator with example.

Allosteric activators are molecules that bind to a regulatory site (other than the active site) of an enzyme and increase its activity by enhancing substrate binding or catalytic efficiency. Common allosteric activators include ATP, which activates phosphofructokinase-1 (PFK-1) in some tissues; AMP, an important activator of PFK-1 in glycolysis; fructose-2, 6-bisphosphate, a powerful allosteric activator of PFK-1; acetyl-CoA, which activates pyruvate carboxylase; and citrate, which activates acetyl-CoA carboxylase in fatty acid synthesis.

6. How ATP signaling feedback glycolysis of PFK-1 phosphorylation?

ATP regulates glycolysis by feedback signaling at the level of phosphofructokinase-1 (PFK-1), the key rate-limiting enzyme of the pathway. When cellular ATP levels are high, ATP binds not only to the active site of PFK-1 as a substrate but also to a distinct allosteric regulatory site. Binding of ATP to this allosteric site induces a conformational change that decreases the enzyme's affinity for fructose-6-phosphate, thereby inhibiting its activity. As a result, the conversion of fructose-6-phosphate to fructose-1,6-bisphosphate slows down, reducing the overall rate of glycolysis. This negative feedback mechanism ensures that glycolysis is downregulated when the cell has sufficient energy, while low ATP (and high AMP or ADP) relieves inhibition and allows glycolysis to proceed.

7. How GTP links with ATP in the Kreb's cycle?

When the cell has plenty of ATP, it signals that energy demand is low. ATP then binds to PFK-1 at a regulatory (allosteric) site, different from the active site. This binding changes the enzyme's shape and reduces its affinity for fructose-6-phosphate, slowing the reaction that commits glucose to glycolysis. As a result, glycolysis is down-regulated. When ATP levels fall and AMP or ADP rise, these molecules bind to PFK-1 and reverse ATP inhibition, activating the enzyme. This allows glycolysis to speed up and generate more ATP. In addition, ATP indirectly influences PFK-1 through PFK-2/FBPase-2, which controls levels of fructose-2,6-bisphosphate, a strong activator of PFK-1. High ATP favors conditions that lower fructose-2,6-bisphosphate, further slowing glycolysis.

8. Why heart run in TCA at high steady rate?

The heart runs the TCA cycle at a high and steady rate because it has a continuous and very high demand for energy to maintain rhythmic and uninterrupted contraction. Cardiac muscle depends almost entirely on aerobic metabolism, and therefore requires a constant supply of ATP. The TCA cycle operates continuously to provide reduced coenzymes (NADH and $FADH_2$) for the electron transport chain, which generates ATP through oxidative phosphorylation.

In addition, the heart has a high mitochondrial density and abundant oxygen supply, allowing the TCA cycle to proceed efficiently at all times. The cardiac muscle can oxidize multiple substrates such as fatty acids, glucose, lactate, and ketone bodies, all of which feed into the TCA cycle, ensuring a steady flux. Because the heart has minimal capacity for anaerobic glycolysis and limited energy storage, it relies on a continuously active TCA cycle to meet its constant energy needs.

9. What happens if proton gradient is not formed in ATP synthesis?

If a proton gradient is not formed across the inner mitochondrial membrane, ATP synthesis cannot occur efficiently. Here's why:

- The proton gradient (proton motive force) generated by the electron transport chain (ETC) is the driving force for ATP synthase to phosphorylate ADP to ATP.
- Without this gradient, protons cannot flow back through ATP synthase, so the enzyme cannot produce ATP.
- Electron transport may continue, but the energy released is dissipated as heat instead of being stored as chemical energy.
- This situation can occur in the presence of uncouplers (e.g., DNP), damage to the mitochondrial membrane, or defective ETC complexes.
- As a result, the cell experiences energy deficiency, which can impair all ATP-dependent processes like muscle contraction, active transport, and biosynthesis.

10. Write role of external and internal signals in metabolic pathway.

Role of External and Internal Signals in Metabolic Pathways

External signals:

- These come from the environment or other cells and help the cell adjust its metabolism according to conditions.
- Examples include hormones like insulin, glucagon, and epinephrine, which regulate carbohydrate, fat, and protein metabolism.
- Nutrient availability (like glucose or amino acids) also acts as an external signal, turning pathways on or off.
- External signals mainly work through receptor-mediated pathways that activate or inhibit enzymes in metabolic pathways.

Internal signals:

- These are molecules within the cell that indicate the cell's energy status or metabolite levels.
- Examples include ATP, ADP, AMP, NADH, acetyl-CoA, and citrate.
- High ATP or NADH often inhibits energy-producing pathways (like glycolysis or TCA cycle), while high ADP/AMP activates them.
- Internal signals act via allosteric regulation, covalent modification (like phosphorylation), or gene expression changes to maintain metabolic balance.

11. How mitochondrial structure important in proton gradient?

The mitochondrial structure is crucial for establishing and maintaining the proton gradient, which drives ATP synthesis. Key points:

- ***Inner mitochondrial membrane (IMM):*** Highly folded into cristae, which increase surface area for electron transport chain (ETC) complexes and ATP synthase. This allows more protons to be pumped and more ATP to be produced.
- ***Impermeability of IMM:*** The inner membrane is impermeable to protons, ensuring that protons accumulate in the intermembrane space instead of leaking back into the matrix. This creates a strong electrochemical gradient (proton motive force).
- ***Matrix compartment:*** Houses enzymes of the TCA cycle that generate NADH and $FADH_2$, which feed electrons into the ETC, initiating proton pumping.
- ***Intermembrane space:*** Serves as a proton reservoir, so the gradient can be effectively used by ATP synthase for phosphorylation of ADP to ATP.

12. What is role of active site in catalyst and substrate recognition?

The active site of an enzyme plays a central role in both catalysis and substrate recognition:

- ***Substrate recognition:*** The active site has a specific shape and chemical environment that matches the substrate (like a lock and key or induced fit). This ensures high specificity, so the enzyme binds only its intended substrate.
- ***Catalysis:*** Once the substrate binds, the active site positions it correctly and provides functional groups that stabilize the transition state, lower the activation energy, and facilitate the chemical reaction.

13. Explain relationship between ΔG and Keq.

The relationship between ΔG (Gibbs free energy change) and Keq (equilibrium constant) shows how the spontaneity of a reaction relates to its position at equilibrium.

- $\Delta G^{\circ\prime}$ represents the standard free energy change under standard conditions, while Keq indicates the ratio of products to reactants when the reaction is at equilibrium.
- If $\Delta G^{\circ\prime}$ is negative, the reaction is spontaneous, and products are favored, so Keq is greater than 1.
- If $\Delta G^{\circ\prime}$ is positive, the reaction is non-spontaneous, reactants are favored, and Keq is less than 1.
- If $\Delta G^{\circ\prime}$ is zero, the reaction is at equilibrium, and Keq equals 1.

14. How ATPs link anabolic and catabolic reactions?

ATP links anabolic and catabolic reactions by acting as the universal energy currency of the cell:

- Catabolic reactions (like glycolysis, TCA cycle, and fatty acid oxidation) break down nutrients and release energy, which is captured in the form of ATP.
- Anabolic reactions (like protein, lipid, and nucleic acid synthesis) require energy to build complex molecules from simpler precursors. ATP provides this energy by donating a phosphate group or transferring high-energy electrons.
- In this way, ATP couples energy release and energy consumption: energy produced in catabolism is stored in ATP, which is then used immediately or later to drive anabolic processes.
- This coupling maintains metabolic balance and efficiency, ensuring that energy is neither wasted nor depleted.

15. How helper play their role in complex enzymes system?

Helpers in complex enzyme systems are usually called coenzymes or cofactors, and they are essential for enzyme function:

- Cofactors can be metal ions (like Mg^{2+}, Zn^{2+}, Fe^{2+}) that help stabilize the enzyme or participate in catalysis.
- Coenzymes are organic molecules (like NAD^{+}, FAD, CoA, or biotin) that temporarily carry chemical groups or electrons during the reaction.
- They assist the enzyme by:
    - Stabilizing the substrate or enzyme structure
    - Participating directly in the chemical reaction
    - Transferring electrons, atoms, or functional groups between molecules
- Some coenzymes act as **"**helper molecules**"** that shuttle energy or intermediates from one reaction to another, increasing the efficiency of multi-step pathways.

16. State one anabolic reaction of TCA in liver.

One anabolic reaction of the TCA cycle in the liver is the production of oxaloacetate from pyruvate via pyruvate carboxylase.

- This reaction provides oxaloacetate, a key precursor for gluconeogenesis, allowing the liver to synthesize glucose from non-carbohydrate sources.
- It links the TCA cycle to glucose production, an essential anabolic pathway, especially during fasting.

17. What is cofactor and coenzyme? Write with examples.

Cofactor:

- A cofactor is a non-protein chemical element or ion required for the activity of an enzyme.
- Cofactors help enzymes stabilize structures or participate directly in catalysis.
- ***Examples*:** Mg^{2+} (required for kinases), Zn^{2+} (required for carbonic anhydrase), Fe^{2+}/Fe^{3+} (required for cytochromes).

Coenzyme:

- A coenzyme is an organic molecule that binds to an enzyme and helps carry chemical groups, electrons, or atoms during the reaction.
- Coenzymes often act as transient carriers and are usually derived from vitamins.
- ***Examples:*** NAD^+ (electron carrier), FAD (electron carrier), CoA (acyl group carrier), biotin (CO_2 carrier in carboxylation reactions).

18. Why do RBCs rely on glycolysis for ATP?

Red blood cells (RBCs) rely on glycolysis for ATP because they lack mitochondria. Without mitochondria, RBCs cannot perform oxidative phosphorylation or the TCA cycle, so glycolysis is their only source of energy.

- ATP produced via glycolysis is used to maintain ion gradients (like Na^+/K^+ pumps) and membrane integrity, which are essential for RBC survival and flexibility.
- RBCs also generate 2,3-bisphosphoglycerate (2,3-BPG) via glycolytic intermediates, which regulates oxygen release from hemoglobin.

19. Compare the ATP yield from glycolysis vs glycolysis oxidation.

1. Glycolysis alone (anaerobic conditions):

- Glucose → 2 pyruvate
- Net ATP produced directly: 2 ATP per glucose
- No oxygen required
- Produces 2 NADH, but without mitochondria or oxygen, NADH cannot be fully oxidized, so ATP yield stays at 2 ATP.

2. Glycolysis with complete oxidation (aerobic conditions):

- Glucose → 2 pyruvate → Acetyl-CoA → TCA cycle → ETC
- ATP from glycolysis: 2 ATP
- ATP from NADH produced in glycolysis (via shuttle into mitochondria): ~3–5 ATP
- ATP from TCA cycle + ETC: ~28–30 ATP per glucose
- Total ATP yield: ~30–32 ATP per glucose
- Much more efficient due to oxidative phosphorylation.

20. Define fermentation and its role in food processing.

Fermentation is a metabolic process in which microorganisms, such as bacteria, yeast, or fungi, convert organic compounds (usually sugars) into simpler products like alcohol, acids, or gases in the absence of oxygen. It allows cells to regenerate NAD^+ for glycolysis, enabling ATP production under anaerobic conditions.

Role in food processing:

- ***Preservation:*** Fermentation produces acids (like lactic acid) or alcohol that inhibit spoilage microorganisms.
- ***Flavor and texture:*** It develops characteristic taste, aroma, and texture in foods like yogurt, cheese, sourdough bread, and kimchi.
- ***Nutritional enhancement:*** Fermentation can increase vitamin content (e.g., B vitamins) and digestibility of foods.
- ***Alcohol production:*** Used in making beer, wine, and spirits by yeast fermentation.

21. What happen in glycolysis in liver when cAMP level rises?

When cAMP levels rise in the liver, it triggers hormone-mediated signaling (usually via glucagon or epinephrine) that leads to inhibition of glycolysis. Here's what happens:

- Increased cAMP activates protein kinase A (PKA).
- PKA phosphorylates key glycolytic enzymes, especially phosphofructokinase-2 (PFK-2)/fructose-2,6-bisphosphatase, converting it to the form that breaks down fructose-2,6-bisphosphate.
- Fructose-2,6-bisphosphate is a potent activator of PFK-1, the rate-limiting glycolytic enzyme. Its reduction decreases PFK-1 activity.
- As a result, glycolysis slows, and glucose is spared for gluconeogenesis and blood glucose maintenance.

22. Explain Positive and Negative Feedback.

Feedback regulation is a mechanism where the **e**nd product of a pathway regulates its own production by affecting an earlier step. There are two types:

1. Positive Feedback:

- The end product or a downstream intermediate enhances the activity of an enzyme or pathway.
- This amplifies the process, often leading to rapid responses.
- ***Example:*** In blood clotting, thrombin activates factors that generate more thrombin, accelerating clot formation.

2. Negative Feedback:

- The end product inhibits an enzyme that acts earlier in the pathway.
- This prevents overproduction, maintaining homeostasis.
- ***Example:*** ATP inhibits phosphofructokinase-1 (PFK-1) in glycolysis. High ATP levels signal that energy is sufficient, slowing down glucose breakdown.

23. Describe the different roles of chemiosmosis.

Chemiosmosis is the process by which protons (H^+) move across a membrane down their electrochemical gradient, driving the synthesis of ATP. Its roles include:

1. ***ATP production:*** Protons pumped into the intermembrane space by the electron transport chain flow back into the mitochondrial matrix through ATP synthase, providing the energy to convert ADP to ATP.
2. ***Coupling electron transport to phosphorylation:*** Chemiosmosis links the energy released from electron transport to ATP synthesis, ensuring efficient energy conversion.
3. ***Maintaining proton motive force:*** The proton gradient not only drives ATP production but also stores potential energy that can be used for other processes.
4. ***Transport across membranes:*** The proton gradient can be used for active transport of metabolites, such as phosphate, pyruvate, and calcium ions, into or out of mitochondria or chloroplasts.
5. ***Heat generation:*** In some cases (like in brown adipose tissue), chemiosmosis can release energy as heat instead of ATP, contributing to thermoregulation.

24. Describe the role of helpers in enzyme activity like pyruvate dehydrogenase.

In complex enzyme systems like **pyruvate dehydrogenase (PDH)**, **helpers**—coenzymes and cofactors—are essential for catalysis. PDH is a multi-enzyme complex that converts **pyruvate to acetyl-CoA**, linking glycolysis to the TCA cycle.

Roles of helpers in PDH:

1. ***Thiamine pyrophosphate (TPP):*** Derived from vitamin B1, TPP binds to the decarboxylation site of pyruvate, helping remove CO_2 and stabilize the resulting hydroxyethyl group.
2. ***Lipoic acid (lipoamide):*** Acts as a swinging arm to transfer the hydroxyethyl group from TPP to CoA, forming acetyl-CoA, and also participates in redox reactions.
3. ***Coenzyme A (CoA):*** Accepts the acetyl group to form acetyl-CoA, which enters the TCA cycle.
4. ***FAD (flavin adenine dinucleotide):*** Accepts electrons during the regeneration of lipoamide and becomes $FADH_2$.
5. ***NAD^+ (nicotinamide adenine dinucleotide):*** Accepts electrons from $FADH_2$, forming NADH, which feeds into the electron transport chain for ATP production.

25. Name the disease that causes the defect of mutation & explain why this happening so.

A classic example is sickle cell anemia:

- ***Cause:*** It is caused by a point mutation in the β-globin gene, where the amino acid glutamic acid is replaced by valine at position 6 of the hemoglobin protein.
- ***Effect:*** This single change alters the hemoglobin structure, making it **s**tick together under low oxygen conditions, which deforms red blood cells into a sickle shape.
- ***Why it happens:*** The mutation changes the chemical properties of hemoglobin, causing it to polymerize, block small blood vessels, reduce oxygen transport, and lead to symptoms like anemia, pain, and organ damage.

26. Write role of ATP in brain cell and liver cell.

Role of ATP in brain cells:

- Provides energy for neuronal signaling, including the generation and propagation of action potentials via Na^+/K^+ pumps.
- Powers neurotransmitter synthesis, release, and reuptake at synapses.
- Supports cellular maintenance, such as protein synthesis, axonal transport, and ion balance.
- Since neurons have limited energy storage, ATP must be continuously produced via glucose metabolism.

Role of ATP in liver cells:

- Drives biosynthetic (anabolic) processes, such as gluconeogenesis, urea synthesis, and lipid synthesis.
- Powers detoxification reactions in the liver, including conjugation of drugs and metabolites.
- Supports active transport of ions and molecules into and out of cells.
- Provides energy for glycogen synthesis and breakdown, helping regulate blood glucose levels.

27. Write role of fermentation in food industry.

Role of Fermentation in the Food Industry:

- ***Preservation:*** Fermentation produces acids (like lactic acid) or alcohol, which inhibit the growth of spoilage microbes, extending shelf life.
- ***Flavor and Texture Development:*** Microbial fermentation generates distinct flavors, aromas, and textures in foods such as yogurt, cheese, kimchi, and sourdough bread.
- ***Nutritional Enhancement:*** Fermentation can increase vitamin content (e.g., B vitamins) and improve digestibility of proteins and carbohydrates.
- ***Alcohol Production:*** Yeast fermentation converts sugars into ethanol and CO_2, producing beer, wine, and spirits.
- ***Functional Foods and Probiotics:*** Fermentation introduces beneficial bacteria into foods, promoting gut health.

28. Write effects of competitive and non-competitive inhibitors when substrate amount is high.

Effects of inhibitors when substrate concentration is high:

1. Competitive Inhibitors:

- They compete with the substrate for binding at the enzyme's active site.
- When substrate concentration is high, the substrate can outcompete the inhibitor, so the enzyme activity can approach normal levels.
- Effect: Inhibition is reduced; Vmax remains the same, but apparent Km increases.

2. Non-Competitive Inhibitors:

- They bind to a site other than the active site and do not compete with the substrate.
- Increasing substrate concentration does not overcome inhibition.
- Effect: Enzyme activity remains inhibited; Vmax decreases, but Km stays the same.

29. Why cancer tissue uses glycolysis continuously even in the presence of oxygen?

Cancer cells exhibit a phenomenon called the Warburg effect, where they rely on glycolysis for energy production even when oxygen is abundant. The reasons are:

- ***Rapid energy supply:*** Glycolysis provides ATP faster than oxidative phosphorylation, which supports the high energy demand of rapidly dividing cancer cells.
- ***Biosynthetic precursors:*** Intermediates of glycolysis are diverted into nucleotide, amino acid, and lipid synthesis, which are essential for cell proliferation.
- ***Hypoxic tumor regions:*** Tumor tissues often have low oxygen areas, so glycolysis ensures continuous ATP production even under fluctuating oxygen levels.
- ***Avoiding oxidative stress:*** Relying less on mitochondria reduces reactive oxygen species (ROS) production, which can damage DNA and proteins.

30. Non-competitive inhibition is not effected even when substrate concentration is increased. Why?

Non-competitive inhibition is not affected by increasing substrate concentration because the inhibitor binds to a site other than the enzyme's active site (an allosteric site).

- This binding changes the enzyme's shape or alters the active site so that the enzyme cannot effectively catalyze the reaction, even if there is plenty of substrate.
- Since the inhibitor does not compete with the substrate for the active site, adding more substrate cannot overcome the inhibition.
- Effectively, Vmax decreases, but Km (substrate affinity) remains unchanged.

31. Write four ETC and their general role.

The Electron Transport Chain (ETC) consists of four major protein complexes in the inner mitochondrial membrane, each with a specific role in energy production:

1. **Complex I (NADH: ubiquinone oxidoreductase):**
   - Accepts electrons from **NADH** and transfers them to **ubiquinone (coenzyme Q)**.
   - Pumps **protons (H^+) into the intermembrane space**, contributing to the proton gradient.
2. **Complex II (Succinate dehydrogenase):**
   - Accepts electrons from **$FADH_2$** generated in the TCA cycle and transfers them to **ubiquinone**.
   - Does **not pump protons**, but feeds electrons into the chain.
3. **Complex III (Ubiquinone: cytochrome c oxidoreductase):**
   - Transfers electrons from **ubiquinol (reduced coenzyme Q)** to **cytochrome c**.
   - Pumps **protons into the intermembrane space**, strengthening the proton gradient.
4. **Complex IV (Cytochrome c oxidase):**
   - Transfers electrons from **cytochrome c** to **molecular oxygen**, reducing it to water.
   - Pumps additional **protons**, finalizing the proton gradient used for ATP synthesis.

General role of ETC:

- Transfers electrons from NADH and $FADH_2$ to oxygen.
- Creates a **proton gradient** across the inner mitochondrial membrane.
- Provides the **energy needed for ATP synthesis** via chemiosmosis.

32. Define hydrolysis of ATP and its products.

Hydrolysis of ATP is the breakdown of adenosine triphosphate (ATP) by water to release energy that the cell can use.

Products of ATP hydrolysis:

1. ***ADP (adenosine diphosphate):*** The molecule left after one phosphate group is removed.
2. ***Inorganic phosphate (Pi):*** The phosphate group released during hydrolysis.
3. ***Energy:*** Free energy released is used to power cellular activities such as muscle contraction, active transport, and biosynthesis.

33. Define standard free energy and its example from a biochemical reaction.

Standard free energy (ΔG°′) is the **ch**ange in Gibbs free energy of a reaction under standard biochemical conditions, which include: pH 7, 1 M concentrations of reactants and products, 25°C (298 K), and 1 atm pressure. It indicates whether a reaction is spontaneous (negative ΔG°′) or non-spontaneous (positive ΔG°′) under these conditions.

Biochemical example:

The hydrolysis of ATP to ADP and inorganic phosphate has a ΔG°′ of approximately −7.3 kcal/mol, showing that it is a spontaneous reaction and a major source of cellular energy.

34. What is gluconeogenesis? Write any three precursors of it.

Gluconeogenesis is the metabolic process of synthesizing glucose from non-carbohydrate precursors, mainly occurring in the liver and kidney. It helps maintain blood glucose levels during fasting or intense exercise.

Three precursors of gluconeogenesis:

1. ***Lactate*** – produced from anaerobic glycolysis in muscles and red blood cells.
2. ***Glycerol*** – derived from the breakdown of triglycerides in adipose tissue.
3. ***Amino acids*** *(mainly* ***alanine****)* – derived from protein catabolism.

35. Justify the statement "Uncoupler are always harmful" by reference of physiological and clinical point of view.

The statement **"**Uncouplers are always harmful**"** is justified from both physiological and clinical perspectives:

Physiological point of view:

- Uncouplers, like 2,4-dinitrophenol (DNP), dissipate the proton gradient across the mitochondrial inner membrane.
- This prevents ATP synthase from producing ATP, even though electron transport continues.
- As a result, cells cannot generate sufficient ATP, which is essential for vital functions like muscle contraction, nerve signaling, and biosynthesis.
- The energy from the proton gradient is released as heat, which can lead to hyperthermia.

Clinical point of view:

- Exposure to uncouplers can cause severe energy depletion, organ failure, and potentially death.
- Hyperthermia and uncontrolled metabolism due to uncouplers have been reported in DNP poisoning.
- No safe therapeutic window exists for most chemical uncouplers in humans; even small doses can be toxic.