Bioenergetics - BCH 314

Unit: 1

Course Outline

The term Bioenergetics
Laws of Thermodynamics
Thermodynamics of ions transport (Chemical potentials and electrochemical potentials)
Energy-rich compounds
Energy-drive reactions in organisms
Oxidative phosphorylation and electron transport chain
Regulation of ATP production
Membrane transport systems

Bioenergetics

- Bioenergetics, also known as biochemical energetics, is the study of energy transformation in living cells.
- Energy is being transformed within cells, from one energy-rich compound to another (e.g. from ATP to ADP and vise-versal; ADP to AMP and vise-versal; G-3-P to 1,3-bisphosphoglycerate, etc.).
- Energy transformation is important because all living things require a continuous supply of energy to stay alive, grow and reproduce.
- The transformation of energy is called **Thermodynamics.**
- **What is Thermodynamics?**

(Thermo = heat, dynamics = power)

Thermodynamics is a branch of science that deals with the transformation or interconversion of different forms of energy, and how the energy is utilized.

NOTE: Forms of energy include:

Light (radiant) energy

Thermal (Heat) energy

Chemical energy

Electrical energy

Mechanical energy

Laws of Thermodynamics

- There are three known laws for thermodynamics (1st, 2nd, and 3rd laws).

The first law of thermodynamics (Principle of energy conservation) states that energy can neither be created nor destroyed but can be transformed from one form to another (therefore, the total energy of a universe or an isolated system is constant).

By mathematical expression, it implies that heat content is the sum of internal energy and work-done.

$H = E + PV$ OR **$q = U + PV$**

$\Delta H = \Delta E + \Delta PV$ **$\Delta q = \Delta U + \Delta PV$**

$\Delta E = \Delta H - w$ **$\Delta U = \Delta q - w$**

Where H or q = Enthalpy/heat (energy) content/ total energy in calmol^{-1}, E or U = internal energy/energy within the system.

P = pressure exerted on the system; V = volume of the system; PV = w = work-done on the system by the surroundings.

When H = E, no work is done but when E < H, work is done

For example, calculate the internal energy in a reaction catalyzed by hexokinase as given below:

Glucose +MgATP ⟷ Glucose-6-phosphate +MgADP. Given that the energy content is 31.2 calmol^{-1} and workdone is 18.05 calmoml^{-1}.

Using **$\Delta E = \Delta H - w$, ΔE =**31.2 calmol^{-1}**, w =** 18.05 calmol^{-1}**, therefore,** 31.2 -18.05 = 13.15 calmol^{-1}. Since $\Delta E < \Delta H$, work is done.

Terminologies in Thermodynamics

System: is a part of the universe that is under study. E.g. organelle, cell, tissue, organism.

Surroundings: is a part of the universe that is not under study.

Open system: is a thermodynamic system that can exchange both matter and energy with its surroundings.

Closed system: is a thermodynamic system that can exchange energy but not matter with its surroundings.

Isolated system: A thermodynamic system that can neither exchange matter nor energy with its surroundings.

Adiabatic process: is a thermodynamic process that is thermally insulated from its surroundings. No heat loss/gain.

Isochoric process: is a thermodynamic process that occurs at a constant volume.

Isothermal process: is a thermodynamic process that occurs at a constant temperature.

Isobaric process: is a thermodynamic process that occurs at a constant pressure.

Enthalpy (H): is the heat/energy content of a reaction/system ($calmol^{-1}$)

NOTE:

When ΔH is negative, the process produces heat and is termed exothermic.

When ΔH is positive, it absorbs heat and is termed endothermic.

Entropy (S): is the quantitative expression of the degree of randomness or disorderliness of a system. It is the amount of energy not available to do work ($calmol^{-1}K^{-1}$).

NOTE:

When ΔS is negative, then the disorder of the system has decreased, i.e., ΔS < 0 (disorder is unfavourable but order is favourable)

When ΔS is positive, then the disorder of the system has increased, i.e., ΔS > 0 (disorder is favourable but order is unfavourable)

The second law of thermodynamics (Principle of direction of the energy conservation) states that all spontaneous processes increase entropy (disorder) of the universe until equilibrium is attained.

By mathematical expression,

$$\Delta S_{system} + \Delta S_{surroundings} = \Delta S_{universe} > 0$$

The third law of thermodynamics (Principle relating to the magnitude of entropy) states that the entropy of a pure and perfectly crystalline substance (perfect order) is 0 at absolute temperature (0K).

In other words, it states that the entropy of a system approaches a constant value as the temperature approaches absolute temperature (0K). Though it failed to tell us how fast or slow the reaction will occur.

What is Gibbs free energy (G)?

Gibbs free energy is the total amount of energy that is available to do work during a process at a constant temperature and pressure.

NOTE:

If ΔG is negative, then the process is spontaneous and is termed exergonic.

If ΔG is positive, then the process is nonspontaneous and is termed endergonic.

If ΔG is zero, then the process has reached equilibrium.

Thermodynamics of Ion Transport

- Free energy is minimized when all concentrations are equal. In other words, concentration gradient (an unequal distribution of molecules) requires an input of free energy (… is an active transport).
- The free energy-change in transporting a solute molecule (uncharged) or ion (charged) from side 1 (where it is present at C_1), to side 2 (where it is present at C_2) is given by the equations below:

a. Energetic cost (ΔG) for moving uncharged solute molecule, which is also known as chemical potential implies that:

$\Delta G_{1\rightarrow 2} = RT\text{Ln}\,(C_2/C_1)$ **OR** $\Delta G_{2\rightarrow 1} = RT\text{Ln}\,(C_1/C_2)$

b. Energetic cost (ΔG) for moving charged molecule (ion), which is also known as electro chemical potential implies that:

$\Delta G_{1\rightarrow 2} = RT\text{Ln}\,(C_2/C_1) + zF\Delta\Psi$ **OR** $\Delta G_{2\rightarrow 1} = RT\text{Ln}\,(C_1/C_2) + zF\Delta\Psi$

(chemical potential + electrical potential)

$\Delta G_{in \rightarrow out} = RTLn\ [K^+])_{out} / [K^+])_{in}$ (chemical potential)

$\Delta G_{out \rightarrow in} = RTLn\ [K^+])_{in} / [K^+])_{out} + zF\Delta\Psi$

(chemical potential + electrical potential) = electrochemical potential

NOTE: When energetic cost is +ve, it needs ATP hydrolysis to drive the movement of the molecule. In other words, the transport is active.

When energetic cost is -ve, it does not need ATP hydrolysis to drive any molecule. In other words, the transport is passive.

Where ΔG = change in Gibbs free energy

R = Gas constant = $1.983 cal mol^{-1} K^{-1}$

T = Temperature in Kelvin (K)

C_2 = Final concentration of the molecule in the 2nd compartment

C_1 = Initial concentration of the molecule in the 1st compartment

z = Number of electrical charges on the charged molecule (ion)

F = Faraday's constant = $23,060 cal mol^{-1}$ or $96,500 J mol^{-1} V^{-1}$

$\Delta\Psi$ = Change in transmembrane electrical potential (V)

NOTE: 1cal = 4.18J

Change in transmembrane electrical potential ($\Delta\Psi$) can be:

$\Delta\psi_{in \rightarrow out} = \Psi_{out} - \Psi_{in}$

$\Delta\psi_{out \rightarrow in} = \Delta\Psi_{in} - \Delta\Psi_{out}$

Calculations

Q1. Consider the transport of an uncharged molecule from $C_1 = 10^{-3}$ to $C_2 = 10^{-1}$, at 25^0C (298K). Given that [R = 1.983calmol^{-1}K^{-1}]. Calculate the change in Gibbs free energy and comment on your answer.

Q2. Calculate the free energy change for glucose entry into the cells when the extracellular concentration is 5mM and the intracellular concentration is 2mM. Given that [R = 1.983calmol^{-1}K^{-1} , T = 25^0C]. Comment on your answer.

Q3. Calculate the free energy difference at 25^0C due to a galactose gradient across a membrane, if the concentration on side 1 is 2mM and the concentration on side 2 is 10mM. Given that [R = 1.983calmol^{-1}K^{-1} , T = 25^0C]. Comment on your answer.

Q4. a. Calculate the chemical potential difference for transporting Na^+ into the cell at 37^0C when intracellular [Na^+] = 10mM and extracellular [Na^+] = 180mM at 37^0C.

b. Calculate the electrical potential difference if the membrane potential were – 60mV (inside negative)

c. What would the electrochemical potential be from the above-given data?[Given that 1F = 23,060calmol^{-1}V^{-1}, R = 1.983calmol^{-1}K^{-1}].

Q5. A typical vertebrate cell has a trans-membrane potential of -0.070 V (inside is negative). What is the free-energy change for transporting 1 mole of Na^+ out of the cell into the blood at 37^0C? Assume the concentration of Na^+ inside the cell is 12mM and that in the blood plasma 145mM. [Given that 1F = 23,600calmol^{-1}V^{-1}, R = 1.983calmol^{-1}K^{-1}].

Q6. Consider a phospholipid vesicle containing 10mM Na^+ ions. The vesicle is bathed in a solution that contains 53 mM Na^+, and the electrical potential difference across the vesicle membrane, $\Delta\psi_{in \rightarrow out} = \Psi_{out} - \Psi_{in}$ = -30mV. What is the electrochemical potential at 25^0C for Na^+? [Given that 1F = 23,600calmol^{-1}V^{-1}, R = 1.983calmol^{-1}K^{-1}].

Q7. For the example in Q5 above, calculate the electrochemical potential difference if the membrane potential is +60mV.

Importance of thermodynamics

i. It tells whether a chemical reaction will occur or not.

ii. It tells the direction of a reaction, backward or forward; left or right.

iii. It tells the amount of energy that could be obtained from a reaction.

Energy-rich compounds

- The compounds that contain energy bond(s) and upon hydrolysis, yield a large amount of energy under standard conditions are regarded to as energy-rich compounds.
- Some of these compounds are frequently those in which a phosphate group is removed from them by hydrolysis.
- The energy-rich compounds include:

i. Creatine phosphate or phosphocreatine

ii. 1,3-bisphosphoglycerate

iii. Phosphoenolpyruvate

iv. AcetylCoA

v. SuccinylCoA

vi. Nucleoside-5^1-triphosphates (such as ATP, GTP, UTP & CTP)

vii. Nucleoside-5^1-diphosphates (such as ADP, GDP, UDP & CDP)

viii. Nucleoside-5^1-monophosphates (such as AMP, GMP, UMP & CMP)

ix. The reducing equivalents such as NADH and $FADH_2$

Energy-rich compounds

ATP as a case study...

- ATP, known as Adenosinetriphosphate is a high-energy compound that has many functions in many biological systems (cells, tissues, organisms).
- It can be hydrolyzed to ADP or AMP, and upon the hydrolysis it releases a large amount of free energy which is used to drive a variety of metabolic reactions.
- ATP is the energy currency of the cells that links catabolism with anabolism. It can readily transfer phosphate group to glyceraldehyde-3-P to regenerate 1,3-bisphosphoglycerate (a very high-energy compound)

E.g. ATP + Glyceraldehyde-3-P $\xleftrightarrow{\text{phosphoglycerate kinase}}$ 1,3-bisphosphoglycerate + ADP

Creatinephosphate as a case study...

- Creatinephosphate serves as a storage form of free energy in muscle. It can readily transfer phosphate group to ADP to regenerate ATP, use for a short period during strenous excercise.

E.g. Creatinephosphate (phosphcreatine) + ADP $\xleftrightarrow{\text{Creatine kinase}}$ Creatine + ATP

Energy-driving reactions in organisms

i. Synthetic and catabolic processes: Energy in the form of ATP can be used in synthetic processes, such as gluconeogenesis, and fatty acid synthesis, producing glucose and fatty acids, respectively. Also, in catabolism such as glycolysis, where 2 ATP is used at reaction steps 1 and 3.

ii. The synthesis of metabolites: During phosphorylation of glucose, glucose-6-P is formed. A total of -4.0kcal/mol is involved. However, about 3.3kcal/mol is utilized for the phosphorylation while -7.3kcal/mol is given off during the hydrolysis of ATP.

iii. Motion: ATP is used during contraction and relaxation of muscles. An increase in Ca^{2+} concentration in muscle cells causes myosin, actin, and ATP to react. Thus, driving movement of the skeletal muscles.

iv. Metabolism and detoxification: Processes of removing or inactivating and excreting xenobiotics (drugs, poison or foreign toxic substances) from the body uses energy in the form of ATP.

v. Thermogenesis: Part of the energy derived from ATP hydrolysis is used for the production of heat in the body by metabolic processes, and some physical activities, and some are given off.

vi. Photosynthesis: In plants, the ATP produced during light reaction is used to drive the dark reaction.

vii. Active transport: ATP can be used to maintain ion gradient across membranes. This is often catalyzed by Na^{+}-K^{+} -ATPase, Ca+-ATPase, etc.

viii. Cell division and growth

Oxidative phosphorylation

- Oxidative phosphorylation is the process in which ATP is formed as a result of the transfer of electron(s) from NADH or $FADH_2$ (the electron carriers) to O_2 by a series of electron carriers.
- NADH and $FADH_2$ are formed during some metabolic processes, e.g. glycolysis, beta-oxidation, and TCA cycle.
- The NADH and $FADH_2$ are energy-rich compounds.
- When NADH and FADH2 are used to reduce molecular oxygen to water, a large amount of free energy is liberated, which can be used to generate ATP.
- Oxidative phosphorylation in eucaryotes occurs in the inner mitochondria membrane, in a set of membrane-proteins known as the electron transport chain.
- Oxidative phosphorylation is very much efficient in producing ATP, more than glycolysis or TCA. It's a crucial process for meeting the energy demands of cells in aerobic conditions

Electron transport chain (ETC)

- The electron transport chain comprises an enzymatic series of electron donors and acceptors (also known as protein complexes).
- ETC is where the bulk of ATP production occurs through oxidative phosphorylation.
- Each electron donor passes an electron to a more electronegative acceptor which in turn donates the electron again to another acceptor, a process that continues down the series until electrons are passed to O_2.
- O_2 is the most electronegative and terminal electron acceptor in the chain, it is the final acceptor that accepts electrons and gets reduced to water.
- As electrons are passed from one carrier to another, H^+ is transported into the inner-mitochondria space, creating an electrochemical gradient. This gradient stores potential energy.
- ETC plays a central role in cellular energy production, converting the energy stored in electron carriers into the chemical energy of ATP through a series of redox reactions and proton pumping.
- Via ETC, each NADH yields about 2.5-3 ATP molecules and each $FADH_2$ yields about 1.5-2 ATP molecules.

Components of ETC

1. The protein Complexes: The ETC consists of a series of protein complexes embedded in the inner mitochondrial membrane. These complexes are labeled as Complexes I, II, III, and IV. Each complex contains specific protein subunits and cofactors that facilitate electron transfer.

2. Electron Carriers: Within the complexes, there are electron carriers such as flavoproteins, iron-sulfur proteins, cytochromes, and coenzyme Q (ubiquinone). These carriers alternate between reduced (gaining electrons) and oxidized (losing electrons) states as they shuttle electrons along the chain.

How ETC works

- Electron Input: The ETC begins with the donation of electrons from electron carriers like NADH (from glycolysis and the citric acid cycle) and $FADH_2$ (from TCA) to Complex I or Complex II, respectively.

- Complex I (also known as NADH-CoQ reductase): NADH transfers its electrons to Complex I. This complex pumps protons (H^+) from the mitochondrial matrix into the intermembrane space while passing electrons to ubiquinone (Coenzyme Q).

- Complex II (also known as succinate-CoQ reductase): $FADH_2$ transfers its electrons directly to ubiquinone through Complex II. Unlike complex I, Complex II does not pump protons.

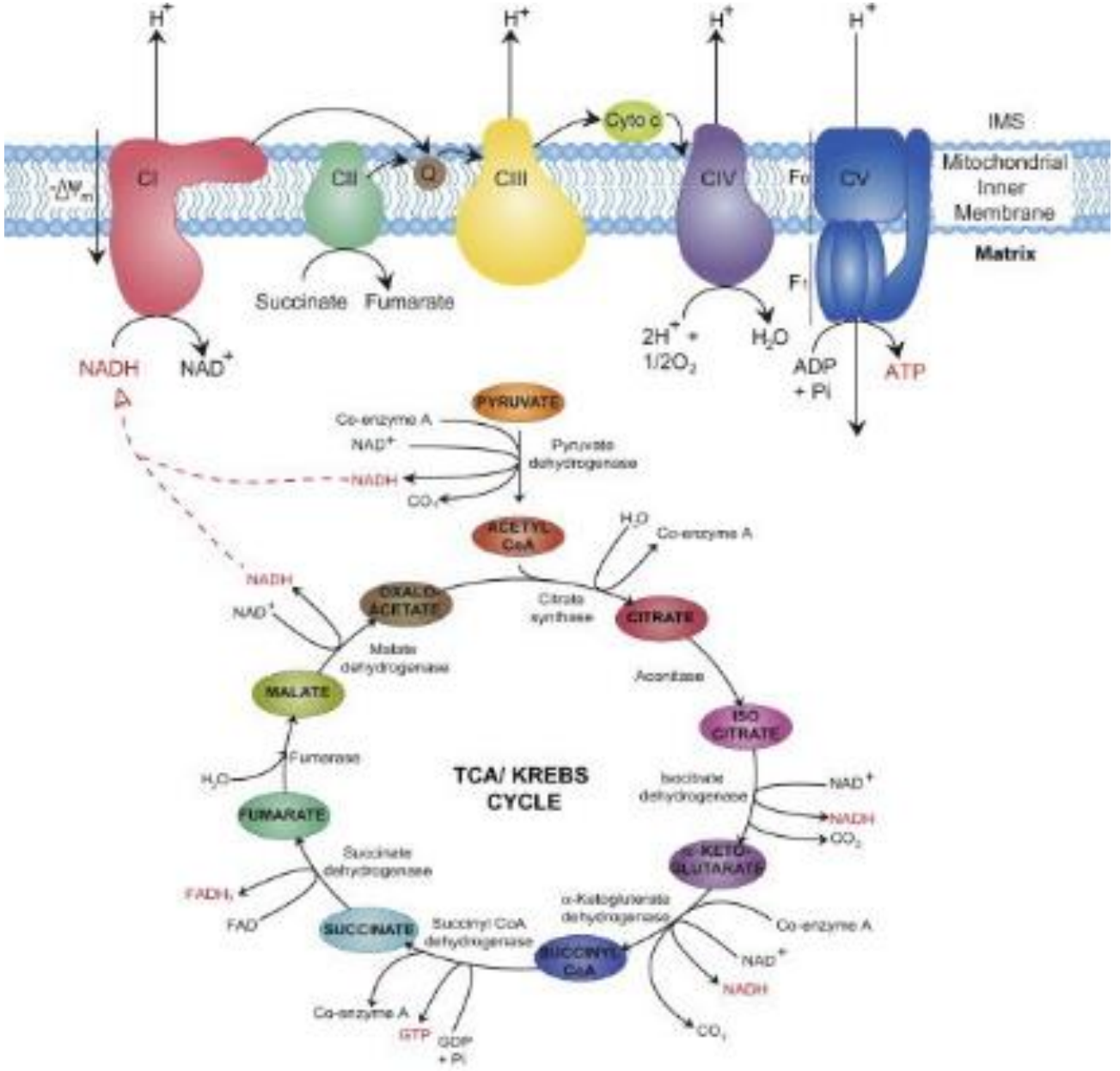
- Coenzyme Q (also known as ubiquinone): Ubiquinone, or Coenzyme Q, serves as a mobile electron carrier. It transports electrons from complexes I and II to Complex III.

- Complex III (also known as CoQ-cytochrome C reductase): Complex III passes electrons from Coenzyme Q to cytochrome C, another mobile carrier protein. Complex III also pumps protons across the inner mitochondrial membrane.

- Cytochrome C: Cytochrome c carries electrons from Complex III to Complex IV.

- Complex IV (also known as cytochrome C oxidase): Complex IV is the final complex in the chain. It transfers electrons from cytochrome C to molecular oxygen (O_2), which serves as the final electron acceptor, forming water (H_2O) in the process. Complex IV also pumps protons across the inner mitochondrial membrane.

Electron-transport chain



Electron transport chain and TCA cycle

Regulation of ATP production

- Regulation of ATP production is a complex process involving multiple factors that include:

i. The levels of ATP in the body
ii. Oxygen concentration
iii. Substrate availability
iv. Hormonal signals
v. Feedback inhibition
vi. Environmental factors

Regulation of ATP production

- ADP and ATP Levels: High levels of ADP indicate a need for ATP synthesis, stimulating the activity of ATP synthase and the ETC to meet cellular energy demands. Otherwise, elevated ATP levels inhibit ATP synthase and reduce ATP production to prevent energy wastage.

- Oxygen availability: Low O_2 levels (hypoxia) can lead to decreased ATP production. The presence of sufficient O_2 is necessary for the electron transport chain to function efficiently, leading to the production of ATP.

- Substrate availability: An abundance of substrates like glucose, acetyl-CoA, and fatty acids, can promote ATP production, and vice versa.

- Hormonal control: Hormones such as insulin and glucagon play key roles in regulating ATP levels. Insulin promotes glucose uptake and glycolysis, leading to increased ATP production, while glucagon stimulates gluconeogenesis and glycogenolysis to release glucose for ATP production.

- Feedback Inhibition: High levels of ATP can inhibit enzymes involved in its synthesis, such as phosphofructokinase in glycolysis and citrate synthase in the citric acid cycle. This feedback inhibition helps prevent excessive ATP production when cellular energy demands are low.

- Environmental factors such as temperature and pH can influence ATP production indirectly by affecting enzyme activity and metabolic rates. Optimal temperature and pH conditions ensure efficient ATP production by maintaining the structural integrity and activity of enzymes involved in energy metabolism.

Transport Across Cell Membrane