BIOENERGETICS

PROF. DR. SHAKIR F. T. ALAARAJI PH. D. BIOCHEMISTRY UNIVERSITY OF ANBAR COLLEGE OF EDUCATION FOR PURE SCIENCES CHEMISTRY DEPARTMENT

- Living organisms must work to stay alive, to grow and to reproduce
- All living organisms have the ability to produce energy and to channel it into biological work
- Living organisms carry out energy transductions, conversions of one form of energy to another form

- Modern organisms use the chemical energy in fuels (carbonhydrates, lipids) to bring about the synthesis of complex macromolecules from simple precursors
- They also convert the chemical energy into concentration gradients and electrical gradients, into motion and heat, and, in a few organisms into light (fireflies, some deep-sea fishes)

- Biological energy transductions obey the same physical laws that govern all other natural processes
- Bioenergetics is the quantitative study of the energy transductions that occur in living cells and of the nature and function of the chemical process underlying these transductions

The goals of this lesson

- Review the laws of thermodynamics
- Understand the quantitative relationships among free energy, enthalpi and entropy
- Describe the special role of ATP in biological energy exchanges

BIOENERGETICS AND THERMODYNAMICS

 Biological energy transductions obey the laws of thermodynamics

Laws of thermodynamics

 1. For any physical or chemical change, the total amount of energy in the universe remains constant; energy may changed from or it may be transported from one region to another, but it can not be created or destroyed 2. The universe always tends toward increasing disorder: in all natural processes the entropy of the universe increases

- The reacting system may be an organism, a cell or two reacting compounds. The reacting system and its surroundings together constitute the universe.
- In the laboratory some chemical or physical processes can be carried out in closed systems and no material or energy is exchanged with the surroundings
- However living organisms are open systems. They exchange both material and energy with their surroundings

• Living systems are never at equilibrium with their surroundings

• Gibbs free energy (G): G expresses the amount of energy capable of doing work during a reaction at constant temperature and pressure.

- When a reaction proceeds with the release of free energy (that is, when the system changes so as to posses less free energy)
 ΔG has a negative value and the reaction is said to be exergonic
- In endergonic reactions, the system gains free energy and ΔG is positive
- The unit of ΔG is joules/mole or calories/mole

- Enthalpy (H): H is the heat content of the reacting system. H reflects the number and kinds of chemical bounds in the reactants and products.
- When a chemical reaction releases heat, it is said to be exothermic, the heat content of the products is less than that of the reactants and ΔH has a negative value
- Reacting systems that take up heat from their surroundings are endothermic and have positive values of ΔH

- The unit of ΔH is joules/mole or calories/mole
- Entropy (S): S is a quantitative expression for the randomness or a disorder in a system

The unit of ΔS is joules/mole. Kelvin

 Under the constant temperature and pressure changes in free energy, enthalpy and entropy in biological systems are related to each other by the equation

• $\Delta G = \Delta H - T \Delta S$

- ΔG= Change in Gibbs free energy of the reacting system (G_{products} G_{reactives})
- $\Delta H=$ Change in enthalpy of the reacting system($H_{products} H_{reactives}$)
- **T**= Absolute temperature
- ΔS = Change in entropy of the reacting system(S_{products} S_{reactives})

 Living organisms preserve their internal order by taking free energy from their surroundings in the form of nutrients or sunlight and returning to their surroundings an equal amount of energy as heat and entropy

Example: The oxidation of glucose

 Aerobic organisms extract free energy from glucose obtained from their surrouindings by oxidizing the glucose with oxygen (also obtained from surroundings). The end products of this oxidation reaction are CO₂ and H₂O and they are returned to the surroundings. At the end of this process, the surroundings undergo an increase in entropy, whereas the organism itself remains in a steady state and no change occurs in its internal order

$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O$

We can represent this schematically as



- Whenever a chemical reaction results in an increase in the number of molecules the entropy of the surroundings increases
- Whenever a solid substance is converted into liquid or gaseous forms the entropy of the surroundings increases. Because this transformations allow the molecule more freedom for movement

Cells require sources of free energy

- Living organisms acquire free energy from nutrient molecules. Cells transform this free energy into ATP and other energy-rich compounds
- They are capable of providing energy for biological work at constant temparature.

 The composition of a reacting system tends to continue changing until equilibrium is reached. At the equilibrium the rates of the forward and revers reactions are equal and no further change occurs in the system. The K_{eq} is defined by the molar concentrations of products and reactants at equilibrium

- When a reacting system is not at equilibrium, the tendency to move toward the equilibrium represents a driving force. The magnitude of this driving force is expressed as free energy change (ΔG).
- Under standard conditions (25°C), when reactants and products are initially at the 1 M concentrations the force driving the system toward equilibrium is defined as the standard free energy change (ΔG⁰)

- By this definiation, standart state for reactions involves [H⁺] = 1M or pH=0.
- However most biochemical reactions occur in well-buffered aqueous solutions near pH=7
- For convenience of calculations, biochemists define a different standard state in which the concentration of [H⁺] is 10⁻⁷ M, and for reactions that involve Mg²⁺ (available in most reactions involving ATP), its concentration in solution is commonly taken to be constant at 1mM

Physical constants based on this biochemical standard state are called standard transformed constants and written as ΔG¹⁰ and K'eq to distinguish them from the untransformed constants which are used by chemists.

- ΔG¹⁰ is the difference between the free energy content of the products and the free energy contents of the reactants under standard conditions
- $\Delta G'^0 = \Delta G'^0_{\text{products}} \Delta G'^0_{\text{reactives}}$)
- When ΔG¹⁰ is negative, the products contain less free energy than the reactants and the reaction will proceed spontaneously under standard conditions
- When ΔG¹⁰ is positive, the products contain more free energy than the reactants and the reaction will tend to go in the revers direction under standard conditions

- Each chemical reaction has a characteristic standard free energy change which may be positive, negative or zero depending on the equilibrium constant of the reaction
- ΔG^{'0} tell us in which direction and how far a given reaction must go to reach equilibrium when the initial concentration of each component is 1M, the pH is 7, the temparature is 25^oC.
- Thus ΔG^{0} is a constant; a characteristic for a given reaction

Actual free energy change

 Actual free energy change (ΔG) is a function of reactant and product concentrations and of the temparature prevailing during the reaction which will not necessarily match the standard conditions as defined before

- ΔG of any reaction proceeding spontaneously toward its equilibrium is always negative, become less negative as the reaction proceeds, and is zero at he point of equilibrium, indicating that no more work can be done by the reaction
- ΔG and $\Delta G^{\prime 0}$ for a reaction like that
- A+B ← C+D
- is written as
- [C] [D]
- $\Delta G = \Delta G'^0 + RT \ln -$

[A] [B]

- An example:
- A+B ← C+D

- Reaction is taking place at the standard temparature and pressure
- But the concentrations of A,B,C and D are not equal and none of them at the 1M concentration
- In order to determine actual ΔG under these non-standard concentrations as the reaction proceeds from left to right, we enter the actual concentrations of A,B,C and D in this equation.

- Rest of the terms in the equation (R,T, ΔG^{0}) are standard values
- When the reaction is at equilibrium there is no force driving the reaction in either direction and ΔG is zero, thus equation reduces to

[C] [D]

• $0 = \Delta G'^0 + RT \ln \theta$

[A] [B]

• $\Delta G^{0} = -RT \ln K' eq \Delta G^{0}$

• The criteria for spontaneity of a reaction is the value of ΔG not ΔG^{10}

- Standard free energy changes are additive.
- In the case of two sequential chemical reactions,



Since the two reactions are sequential, we can write the overall reaction as

• A
$$\longrightarrow$$
 C $\Delta G'_{total}^{0}$

• The ΔG^{0} values of sequential reactions are additive.

$$\Delta G'_{total}^{0} = \Delta G'_{1}^{0} + \Delta G'_{2}^{0}$$



• Sum: $A \longrightarrow C$ $\Delta G^{\prime 0}_{1} + \Delta G^{\prime 0}_{2}$

 This principle of bioenergetics explains how a thermodynamically unfavorable (endergonic) reaction can be driven in the forward direction by coupling it to a highly exergonic reaction through a common intermediate • The main rule in biochemical reactions in living organisms:

 All endergonic reactions are coupled to an exergonic reaction. There is an energy cycle in cells that links anabolic and catabolic reactions.

An example: The first step of glycolysis

Glucose + P_i → Glucose 6-phosphate+H₂O
ΔG^{'0} =13,8 kj/mol

• $\Delta G^{0} > 0$ reaction is not spontaneous

- Another very exergonic cellular reaction:
- Hydrolysis of ATP

• ATP +
$$H_2O \longrightarrow ADP + P_i$$

• $\Delta G'^0 = -30,5 \text{ kj/mol}$

 These two reactions share the common intermediates H₂O and P_i and may be expressed as sequential reactions:
- Glucose + $P_i \longrightarrow Glucose 6$ -phosphate+ H_2O • ATP + $H_2O \longrightarrow ADP + P_i$
- Sum: Glucose+ATP → Glucose 6-phosphate+ADP
- The overall standard free energy changes:
- ΔG'⁰ =13,8 kj/mol + (-30,5 kj/mol)= -16,7
 kj/mol
- Overall reaction is exergonic

- Energy stored in ATP is used to drive to synthesis of glycose 6-phosphate, eventhough its formation from glucose and P_i is endergonic.
- This strategy works only if compounds such as ATP are continuously available.

Phosphoryl group transfer and ATP

- Living cells obtain free energy in a chemical form by the catabolism of nutrient molecules
- They use that energy to make ATP from ADP and $\rm P_{i}$.
- ATP donates some of its chemical energy to
- 1. Endergonic processes such as the synthesis of metabolic intermediates and macromolecules from smaller precursors

- 2. The transport of substances across membranes against concentration gradients
- 3. Mechanical motion (muscle contraction)

 This donation of energy from ATP can occur in the two form

- A) ATP \longrightarrow ADP+ P_i or
- B) ATP → AMP+ 2 P_i

The free energy change for ATP hydrolysis is large and negative

- The hydrolytic cleavage of the terminal phosphoanhydride bond in ATP separates one of the three negatively charged phosphates and thus relieves some of the electrostatic repulsion in ATP
- Released P_i is stabilized by the formation of several resonance forms not possible in ATP

- ADP²⁻ is the other product of hydrolysis and it immediately ionizes, releasing H⁺ into a medium of very low [H⁺] (~10⁻⁷ M)
- Because the concentrations of the products ot ATP hydrolysis are far below the concentrations at equilibrium, mass action favors the hydrolysis in the cell



 Although the hydrolysis of ATP is highly exergonic (ΔG^{'0} = -30,5 kj/mol), the ATP is stable at pH 7, because the activation energy for ATP hydrolysis is relatively high. Rapid hydrolysis of ATP occurs only when catalyzed by an enzyme

- The free energy change for ATP hydrolysis is -30,5 kj/mol under standard conditions but the actual free energy change (ΔG) of ATP hydrolysis in living cells is very different.
- The cellular concentrations of ATP, ADP and P_i are not same and are much lower than the 1 M standard conditions.
- In addition, Mg²⁺ in the cytosol binds to ATP and ADP and for most enzymatic reactions that involve ATP as phosphorly group donor, the true substrate is MgATP⁻². The relevant ΔG^{'0} is therefore that for MgATP⁻² hydrolysis.



FIGURE 13–2 Mg²⁺ and ATP. Formation of Mg²⁺ complexes partially shields the negative charges and influences the conformation of the phosphate groups in nucleotides such as ATP and ADP.

Compounds have large free energy change

- Phosphorylated compounds
- Thioesters (Acetyl-CoA)

Phosphorylated compounds

- Phosphoenolpyruvate
- 1,3-bisphosphoglycerate
- Phosphocreatine
- ADP
- ATP
- AMP
- PPi
- Glucose 1-phosphate
- Fructose 6-phosphate
- Glucose 6-phosphate

Phosphoenolpyruvate

- Phosphoenolpyruvate contains a phosphate ester bond that undergoes to yield to enol form of pyruvate
- The enol form of pyruvate can immediately tautomerize to the more stable keto form of pyruvate. Because phosphoenolpyruvate has only one form (enol) and the product, pyruvate, has two possible forms, the product is more stabilized relative to the reactant.
- This is the greatest contributing factor to the high standard free energy change of hydrolysis of phosphoenolpyruvate ($\Delta G^{0} = -61,9 \text{ kj/mol}$)



 $PEP^{3-} + H_2O \longrightarrow pyruvate^- + P_i^{2-}$ $\Delta G'^{\circ} = -61.9 \text{ kJ/mol}$

1,3-bisphosphoglycerate

- 1,3-bisphosphoglycerate contains an anhydride bond between the carboxyl group at C-1 and phosphoric acid.
- Hydrolysis of this acyl phosphate is accompanied by a large, negative, standard free energy change ($\Delta G^{0} = -49,3 \text{ kj/mol}$)
- This large, negative ΔG¹⁰ can, again, be explained in terms of the structure of reactants and products

- When the water is added to anhyhride bond of 1,3-bisphosphoglycerate, one of the direct products, 3-phosphoglyceric acid, can immediately lose a proton to give the carboxylate ion, 3-phosphoglycerate, which has two equally probable resonance forms
- Removal of a direct product, 3-phosphoglyceric acid, and formation of resonancestabilized ion favor the forward reaction.



Phosphocreatine

• In the phosphocreatine, the P-N bond can be hydrolyzed to generate free creatine and P_i. The release of P_i and the resonance stabilization of creatine favor the forward reaction. The standard free energy change of phosphocreatine is large and negative ($\Delta G'^0 = -49,3$ kj/mol).



FIGURE 13-5 Hydrolysis of phosphocreatine. Breakage of the P—N bond in phosphocreatine produces creatine, which is stabilized by formation of a resonance hybrid. The other product, P_i, is also resonance stabilized.

Phosphocreatine²⁻ + H₂O \longrightarrow creatine + P_i²⁻ $\Delta G'^{\circ} = -43.0 \text{ kJ/mol}$

Thioesters

- In thioesters a sulfur atom is replaced the usual oxygen in the ester bond
- Thioesters have large, negative standard free energy change of hydrolysis.
- Acetyl coenzyme A is one of many thioesters important in metabolism. The acyl group in these compounds is activated for transacylation, condensation or oxidation-reduction reactions.

- Hydrolysis of the ester bond generates a carboxylic acid which can ionize and assume several resonance forms.
- $\Delta G'^0 = -31,4$ kj/mol for acetyl-CoA hydrolysis



FIGURE 13–6 Hydrolysis of acetyl-coenzyme A. Acetyl-CoA is a thioester with a large, negative, standard free energy of hydrolysis. Thioesters contain a sulfur atom in the position occupied by an oxygen atom in oxygen esters. The complete structure of coenzyme A (CoA, or CoASH) is shown in Figure 8–41.

Summary for hydrolysis reactions

- For hydrolysis reactions with large, negative standard free energy changes, the products are more stable than the reactants for one or more of the following reasons:
- 1. The bond strain in reactants due to electrostatic repulsion is relieved by charge separation, as for ATP
- **2.** The products are stabilized by ionization, as for ATP, acyl phosphates, thioesters

- **3.** The products are stabilized by isomerization (tautomerization) as for phosphoenolpyruvate
- 4. The products are stabilized by resonance as for creatine released from phosphocreatine, carboxylate ion released from acyl phosphates and thioesters and phosphate released from anhydride or ester linkages

- The phosphate compounds found in living organisms can be arbitrarily divided into two groups based on their standard free energy changes of hydrolysis.
- 'High-energy' compounds have a ΔG'⁰ of hydrolysis more negative than -25 kj/mol; 'lowenergy' compounds have a less negative ΔG'⁰.
- Based on this criterion, ATP, with a ΔG^{'0} of hydrolysis of -30 kj/mol is a high-energy compound; glucose 6-phosphate is a lowenergy compound (ΔG^{'0} = -13,8 kj/mol)

TABLE 13–6 Standard Free Energies of Hydrolysis of Some Phosphorylated Compounds and Acetyl-CoA (a Thioester)

 $\Delta G'^{\circ}$

	(kJ/mol)	(kcal/mol)
Phosphoenolpyruvate	-61.9	-14.8
1,3-bisphosphoglycerate		
$(\rightarrow 3-phosphoglycerate + P_i)$	-49.3	-11.8
Phosphocreatine	-43.0	-10.3
ADP (\rightarrow AMP + P _i)	-32.8	-7.8
ATP (\rightarrow ADP + P _i)	-30.5	-7.3
ATP ($\rightarrow AMP + PP_i$)	-45.6	-10.9
AMP (\rightarrow adenosine + P _i)	-14.2	-3.4
$PP_i (\rightarrow 2P_i)$	-19.2	-4.0
Glucose 1-phosphate	-20.9	-5.0
Fructose 6-phosphate	-15.9	-3.8
Glucose 6-phosphate	-13.8	-3.3
Glycerol 1-phosphate	-9.2	-2.2
Acetyl-CoA	-31.4	-7.5

Source: Data mostly from Jencks, W.P. (1976) in Handbook of Biochemistry and Molecular Biology, 3rd edn (Fasman, G.D., ed.), Physical and Chemical Data, Vol. I, pp. 296–304, CRC Press, Boca Raton, FL. The value for the free energy of hydrolysis of PP₁ is from Frey, PA. & Arabshahi, A. (1995) Standard free-energy change for the hydrolysis of the α - β -phosphoanhydride bridge in ATP. Biochemistry **34**, 11,307–11,310.

- The term 'high-energy phosphate bond' was used by biochemists to describe P-O bond broken in hydrolysis reactions for a long time. But it is incorrect and misleading as it wrongly suggests that the bond itself contains the energy.
- In fact, the breaking of all chemical bonds requires an input of energy. The free energy released by hydrolysis of phosphate compounds does not come from the specific bond that is broken;

 it results from the products of the reaction having a lower free energy content than the reactants As is evident from the additivity of free energy changes of sequential reactions, any phosphorylated compound can be synthesized by coupling the synthesis to the breakdown of another phosphorylated compound with a more negative standard free energy change of hydrolysis.

- Example:
- PEP + H₂O _____ Pyruvate + P_i -61,9 • ADP+ P_i ATP+ H₂O +30,5
- +
- PEP + ADP Pyruvate + ATP -31,4
- Clevage of P_i from PEP releases more energy than is needed to drive to condensation of P_i with ADP, the direct donation of a phosphoryl group from PEP to ADP is thermodynamically feasible.

- Notice that while the overall reaction above is represented as the algebraic sum of first two reactions, the overall reaction (third) does not involve P_i; PEP donates a phosphoryl group directly to ADP.
- We can describe phosphorylated compounds as having a high or low phosphoryl group transfer potential, on the basis of their standard free energy changes of hydrolysis

- Much of catabolism is directed toward the synthesis of high-energy phosphate compounds, but their formation is not an end in itself; they are the means of activeting a wide variety of compounds for further chemical transformation.
- The transfer of a phosphoryl group to a compound effectively puts free energy into that compound, so that it has more free energy to give up during subsequent metabolic transformations.

- Because of its intermediate position on the scale of group transfer potential, ATP can carry energy from high-energy phosphate compounds produced by catabolism to compounds such as glucose, converting them into more reactive species.
- ATP serves as the universal energy currency in all living cells

- One more chemical feature of ATP is crucial to its role in metabolism: although in aqueous solution ATP is thermodynamically unstable and is therefore a good phosphoryl group donor, it is kinetically stable.
- Because of high activation energies required for uncatalyzed reaction ATP does not spontaneously donate phosphoryl groups to water or to the other potential acceptors in the cell.

- ATP hydrolysis occurs only when specific enzymes which lower the energy of activation are present
- The cell is therefore able to regulate the disposition of the energy carried by ATP by regulating the various enzymes that act on ATP
- Each of the three phosphates of ATP is susceptible to nucleophilic attack and each position of attack yields a different type of product

Three positions on ATP for attack by the nucleophile R¹⁸O



FIGURE 13-10 Nucleophilic displacement read tions of ATP. Any of the three P atoms (α, β, α) may serve as the electrophilic target for nucleophilic attack-in this case, by the labeled nucleophile R—¹⁸O:. The nucleophile may be alcohol (ROH), a carboxyl group (RCOO⁻), or phosphoanhydride (a nucleoside mono- or diphosphate, for example). (a) When the oxygen of the nucleophile attacks the γ position, the bit oxygen of the product is labeled, indicating ha the group transferred from ATP is a phosphon $(-PO_3^{2-})$, not a phosphate $(-OPO_3^{2-})$. (b) Attai on the β position displaces AMP and leads to the transfer of a pyrophosphoryl (not pyrophosphate group to the nucleophile. (c) Attack on the α position displaces PPi and transfers the aden/ group to the nucleophile.
- Attack at the Y phosphate displaces ADP and transfers Pi,
- Attack at the 6 phosphate displaces AMP and transfers PPi,
- Attack at the α phosphate displaces PPi and transfers adenylate
- Notice that hydrolysis of phospho anhydride bond between α and 6 phosphates releases much more energy than hydrolysis of phospho anhydride bond between 6 and ¥ phosphates. Because PPi formed as a by-product of the adenylation is hydrolyzed the two Pi releasing and thereby providing a further energy push for the adenylation reaction

Examples

- Fatty acid activation reactions during the fatty acid synthesis and /or oxidation
- Amino acid activation reactions during the protein synthesis
- DNA and RNA synthesis

Direct hydrolysis of ATP (ATP → ADP+Pi) is the source of energy in the conformational changes that produce muscle contraction and transport an ion or a molecule across a membrane against concentration gradient (An example: Na⁺- K⁺ exchange by Na⁺ K⁺ ATPaz)

Transphosphorylations between nucleotides

 Although we have focused on ATP as the cell's energy currency and donor of phosphoryl groups, all other nucleoside triphosphates (GTP, UTP, CTP) and all the deoxynucleoside triphosphates (dATP, dGTP, dTTP and dCTP) are energetically equivalent to ATP. The free energy changes associated with hydrolysis of their phosphoanhydride linkages are very nearly identical with those for ATP.

- In preparation for their various biological roles, these other nucleotides are generated as the nucleoside triphosphate (NTP) forms by phosphoryl group transfer to the corresponding nucleoside diphosphates (NDPs) and monophosphates (NMPs)
- ATP is the primary high-energy phosphate compound produced by catabolism in the processes of glycolysis, oxidative phosphorylation. Several enzymes carry phosphoryl groups from ATP to the other nucleotides

 Nucleoside diphosphate kinases, found in all cells, catalyzes the reaction

•
$$Mg^{2+}$$

• ATP+NDP(or dNDP) \longrightarrow ADP+NTP(or dNDP)

 Although this reaction is fully reversible the relatively high ATP/ADP ratio in cells normally drives the reaction to the right, with the net formation of NTPs and dNTPs

- Phosphoryl group transfers from ATP result in an accumulation of ADP. For example, when muscle is concracting vigorously ADP accumulates and interferes with ATPdependent contraction.
- During periods of intense demand for ATP, the cell lowers the ADP concentration, and at the same time acquires ATP, by the action of adenylate kinase:

$$Mg^{2+}$$

$$2ADP \xrightarrow{} ATP + AMP$$

- This reaction is fully reversible, so after the intense demand for ATP ends, the enzyme can recycle AMP by converting it to ADP which can then be phosphorylated to ATP in mitochondria
- A similar enzyme guanylate kinase, converts GMP to GDP at the expense of ATP. Bypathways such as these, energy conserved in the catabolic production of ATP is used to supply the cell with all required NTPs and dNTPs

 Phosphocreatine (PCr) serves as a ready source of phosphoryl groups for the quick synthesis of ATP from ADP. The phosphocreatine concentration in skeletal muscle is considerably higher than those in the other tissues. The enzyme creatine kinase catalyzes the reversible reaction.

• Mg²⁺
• ADP+ PCr
$$\leftarrow$$
 ATP+Cr
• $\Delta G'^0 = -12,5$

kj/mol

- When a sudden demand for energy depletes ATP, the PCr reservoir is used to replenish ATP at a rate faster than ATP can be synthesized by catabolic pathways
- When the demand for energy slackens ATP produced by catabolism is used to replenish the PCr reservoir by reversal of the creatine kinase reaction

SUMMARY

- ATP is the chemical link between catabolism and anabolism. The exergonic conversion of ATP coupled to many endergonic processes in living organisms
- Cells also contain some high-energy compounds which have a high phosphorylation potential, like ATP. They are good donors of phosphoryl groups.