INTRODUCTION TO CELL METABOLISM

CITRIC ACID CYCLE

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METABOLISM

= turnover of substances in the organism - chemical (compounds) feature -
reactions - anabolic
- catabolic
- amphibolic
- energetic feature - reactions exergonic: ΔG<0
ΔU(ΔG) ——> work + heat - endergonic: ΔG>0

Anabolic (association, biosynthetic reactions) are *endergonic*,
catabolic (dissociation) reactions are *exergonic*,
amphibolic reactions serve both biosyntheses and biodegradations (citrate Krebs cycle).
***Endergonic* reactions include : -biosyntheses (reduction, condensation),**
-osmotic work (concentration-gradients, ion-gradients),
-mechanical work (muscle contraction - conformation changes of contractile proteins).
***Exergonic* reactions include :**
- *oxidation* (loss of electrons, loss of H atoms, entry of O atoms into the molecule),
- *hydrolytic* reactions, esp. of anhydride bonds (e.g. diphosphates, triphosphates).
ΔG of *oxidation-reduction reaction* (exergonic): n = moles of electrons; *pH*= 7;
$\Delta G' = - n . F . \Delta E'_h$; $F = 96.5\ kJ . V^{-1} . mol^{-1}$; $\Delta E'_h$ = difference in redox-potentials in *V*.
ΔG of *osmotic work* (endergonic): $\Delta G = n . R . T . ln\ c_2/c_1$; $c_2 > c_1$; n = moles of transferred particles; for 37 °C : $\Delta G = n . 5.9 . \log c_2/c_1$ [$kJ . mol^{-1}$]
***Elektrochemical potential (gradient)* for ions (n=1): $\Delta G = R . T . ln\ c_2/c_1 + Z . F . \Delta V$;**
Z = charge of particles;* ΔV = membrane potential in *V

OXIDATION-REDUCTION POTENTIAL (REDOX POTENTIAL) E'_h

is a quantity measuring and expressing the power of a system to donate or accept electrons. A system *donating* electrons functions as a *reducing* half-cell and has a *more negative* redox potential ; a system *accepting* electrons operates as an *oxidizing* half-cell and possesses a more *positive* redox potential. Each half-cell contains a couple of the oxidized and the reduced forms of the respective redox agent or electron carrier (called *redox couple).* Electrons proceed spontaneously from the more negative to the more positive half-cell. During such a transfer they loose part of their energy. The amount of energy released during this spontaneous process (ΔG) is formulated by the redox equation and is proportional to *moles* of transferred *electrons (n)* and to the difference between the redox potentials $\Delta E'_h$ in volts *(V),* multiplied by the joule equivalent of Faraday charge = *(F).* E'_h and $\Delta E'_h$ depend on pH (´means pH 7.0) .

STANDARD REDOX POTENTIALS E_0'

are measured against „normal“ hydrogen electrode under *standard* conditions, where all components are at concentrations 1 mol . l^{-1} and pH of the measured system is 7.0. The respective redox potential E_h' will depend on the *ratio* of the oxidized and reduced forms of both half-cells. *Redox potentials* are measured in *volts (V).*

$$E_h' = E_0' + \frac{R \,.\, T}{n \,.\, F} \ln \frac{[ox]}{[red]}$$

(Nernst-Peters equation)

PURPOSE AND FUNCTION OF METABOLISM

1. To extract energy and/or the reducing power from the *environment*:
-phototrophic organisms from the *photons* (light) by *photosynthesis* (bacteria, plants);
-chemotrophic organisms from the *electrons* by the *oxidation of nutriments* (animals, humans).
2. To employ the *acquired energy* for the biosynthesis of building blocks of *macromolecules* and of macromolecular cell structures themselves.
3. To utilize the *acquired energy* for the conformation changes of *ion pumps* and the conformation changes of *contractile proteins*.
4.These changes must proceed as isothermic processes (e.g. at 37°C).
Mutual conversion of foodstuffs (carbohydrates , fats, proteins) is designated as *intermediary metabolism*. It uses a limited amount of *common intermediates* of metabolism, *activated intermediates (carriers),* a *common* molecular *energetic carrier* (ATP) and a limited amount of typical sequences of reactions - *metabolic pathways,* which may be *regulated* by common or independent *control mechanisms. Employing common mechanisms and intermediates spares the number of required enzymes involved in metabolic processes.*
***Foodstuffs* serve as a source of reducing power (reducing equivalents : electrons, H-atoms, NADPH) and their oxidation may proceed as an anaerobic pathway (*glycolysis)* or an aerobic pathway via electron transfer up to *molecular oxygen in the respiratory chain of the inner mitochondrial membrane (cell respiration).* Molecular oxygen serves as a *terminal electron acceptor* in the reaction:**

$$O_2 + 4\ e^- \longrightarrow 2\ O^{2-}$$

$$2\ O^{2-} + 4\ H^+ \longrightarrow 2\ H_2O$$

During this process CO_2 originates as a side-product in *decarboxylation reactions* and oxygen enters the substrates in the form of water molecules via addition reaction on a double bond. However, the energetic balance of aerobic oxidations results from the transfer of electrons up to the molecular oxygen. Via aerobic degradation of a glucose molecule the yield of ATP is 15 x higher compared to anaerobic glycolysis (the difference in redox potentials of the carriers is greater).

Energy acquired from the environment (from *photons* in *phototrophic* organisms or from *electrons* in *chemotrophic* organisms) may be transformed in the cell:

1. in the form of an *electrochemical ion gradient (e.g. of protons);*
2. forming so called *macroergic (high-energy) compounds,* which are able to shift the chemical equilibrium of coupled reactions in *water* to the right, in favor of the *products* of a coupled reaction (such compounds have a *high group transfer potential* (e.g. for the transfer of such groups as: phosphate-group, acyl-group and others) - *bonds* of such groups are marked ~ (squiggle) ; this transfer represents a *coupling of exergonic and endergonic reactions;*
3. *storing the reducing power* in the form of NADPH

***Standard free* enthalpy change (*standard* Gibbs energy)**

$$\Delta G^{o\prime} = - R \,.T. \ln K_{eq}$$

represents the change in **free** energy (**free** enthalpy), which would occur under ***standard*** *conditions*, i.e. at the concentration of all components 1 mol . l^{-1} and at standard temperature and pressure (´ = pH 7), after reaction of 1 mole of a substance. **Free** energy (free enthalpy) is such, which may **perform work under isothermic conditions .** However**,** energetic balance of a reaction is determined by its **distance from the equilibrium state** resulting from the ***actual* concentrations of reactants and products**. This is expressed by the term ***free*** enthalpy change **ΔG´** .

$$\Delta G' = \Delta G^{o\prime} + R \,.T. \ln \frac{[C] \,.\, [D]}{[A] \,.\, [B]}$$

In a closed system only **exergonic** reactions are spontaneously possible. A highly negative value of **ΔG´** means that the reaction is far from equilibrium, on the side of reactants **[A] a [B] .**

At equilibrium the term **ΔG´** equals **zero.**

$$\Delta G' = \Delta G^{o\prime} + R \,.T. \ln \frac{[C] \,.\, [D]}{[A] \,.\, [B]}$$

At 37 °C and pH 7 it reads:

$$\Delta G' = \Delta G^{o\prime} + 5.9 \,.\, \log \frac{[C] \,.\, [D]}{[A] \,.\, [B]} \quad [kJ.mol^{-1}]$$

$$\Delta G^{o\prime} = - 5.9 \,.\, \log K_{eq} \quad [kJ.mol^{-1}]$$

METABOLISM OPERATES BY THE ACTION OF ENZYMES

1. Enzymes are *tools of gene expression*.
2. Enzymes *combine* various types of *metabolism of foodstuffs* via *common intermediates.*
3. Enzymes enable the existence of some *endergonic* reactions, which would not be spontaneously possible, by their *coupling with exergonic* reactions via *common intermediates*, which makes the process thermodanamically feasible.
4. In the *series of coupled reactions* the *catalytic function* of enzymes to lower the activation energy is *multiplied*, so that the process can be *isothermic*, and being divided in more *partial steps* the released energy may be *utilized with a higher efficiency*.
5.Controlling the *rate of entry and exit* of metabolites in the cells the enzymes operate in an *open system* with its *dynamic equilibrium (steady state*), where *the two rates are equal*, and such a process enables the exergonic reactions to run towards the true (thermodynamic) equilibrium.
6. *Regulation of key enzymes* of metabolic pathways enables *independent control* of catabolic, anabolic, exergonic an endergonic reactions, maintains *metabolic equilibria* and contributes to steady-state equilibria of a *stable internal environment*.
7. *Independent* regulation of *opposite metabolic pathways* is facilitated by their localization in different cell *compartments* (cytoplasm, organelles), cells or organs.

ROLE OF „MACROERGIC“ BONDS

„*Macroergic“ bonds* in some compounds playing key roles in metabolism are marked as ~ (squiggle). This symbol means that the equilibrium constant of the hydrolytic reaction breaking such a bond has a very high value in the order of magnitude 10^4 to 10^5 , thus the respective *standard free enthalpy change of hydrolysis* $\Delta G^{0'}$ represents a value of at least -25 kJ . mol^{-1} (or more negative values). If there are two reactions *coupled* via a *common intermediate*, the *resulting equilibrium constant* of the *coupled reaction* K is a *product* of the *partial* equilibrium constants K_1 and K_2 , thus the *net* $\Delta G^{0'}$ is a *sum* of two *partial* $\Delta G^{0'}$

$$\Delta G^{0'} = \Delta G^{0'}_1 + \Delta G^{0'}_2$$

Following this mechanism a reaction with a *strongly negative* value $\Delta G^{0'}_2$ may more than *compensate* a *positive* value $\Delta G^{0'}_1$, thus the *net* $\Delta G^{0'}$ is *negative* and the reaction is *thermodynamically feasible*. (When multiplying the values K_1 and K_2 , their respective logarithms are summed up.)

E.g.: $-16.5 = +14 - 30.5$ [kJ . mol^{-1}]

„Macroergic“ compounds, or compounds with a *high group transfer potential* (e.g. for a phosphate group transfer), have their equilibrium constants strongly *on the side of the products* and *drive the coupled endergonic reactions* towards the phosphorylated products.

ATP is the universal currency of free enthalpy in biological systems

Anhydride-bonded phosphate groups have a very negative value of standard free enthalpy change of hydrolysis $\Delta G^{0'}$ (a high phosphate group transfer potential):

$$ATP + H_2O \ <=====> \ ADP + P_i \qquad \Delta G^{0'} = -30.5 \text{ kJ . mol}^{-1}$$

Equilibrium constant of this reaction is

$$K = \frac{[ADP] \cdot [P_i]}{[ATP] \cdot [H_2O]} \qquad K = 1.48 \cdot 10^5$$

The above values would hold under *standard* conditions, if concentrations of all components were 1 mol . l^{-1} (for water the activity = 1 is considered). In biological systems it is assumed, that the substance concentration [ATP] exceeds the concentration [ADP] at least 300-times and actual $\Delta G'$ represents the value

$$-50 \text{ kJ . mol}^{-1}$$

FREE ENERGY RELEASED BY CELL OXIDATIONS IS DEPOSITED INTO THE SYSTEM ATP/ADP

ATP may originate by two mechanisms:

1) By so-called *substrate* phosphorylation, where there is an „**macroergic" intermediate** inserted between the oxidation-reduction reaction and the formation of ATP. By this mechanism ATP originates in the anaerobic process of glycolysis in cytoplasm and only in one single reaction of citrate(Krebs) cycle in mitochondria (succinate thiokinase).

2) By the mechanism of *oxidative (aerobic) phosphorylation* via **elektrochemical proton gradient** across the inner mitochondrial membrane, established by proton pumps of oxidation-reduction carriers (complexes). The source of reducing equivalents (electrons and H atoms) are the intermediates of Krebs cycle and beta-oxidation of fatty acids.

ATP IS AN ENERGY-COUPLING AGENT IN THE CELL (COUPLING EXERGONIC AND ENDERGONIC REACTIONS)

Hydrolysis of 1 mole of ATP may change the *equilibrium concentration ratio*:phosforylated products/unphosphorylated reactants in *coupled reactions in the cell* by a factor of about 10^8 .

The *phosphorylated product* may be an *organic substrate* (e.g. glucose), the *protein* of cell membrane Na^+/K^+ *pump* or a *molecular motor* of a contractile protein (e.g. myosin). Phosphorylation of a protein induces a *conformation richer in energy*, which enables the transformation of *chemical energy* of ATP into the formation of *osmotic* (ionic) *gradient* or into the *mechanical energy* (e.g. muscle contraction).

EXCHANGE OF FREE ENTHALPY COUPLED WITH PHOSPHATE GROUPS TRANSFER

Under *standard conditions* (concentration of all components 1 mol . 1^{-1}) the transfer of phosphate groups in water medium proceeds from a system with a *more negative* $\Delta G^{0'}$ to a system with *less negative* $\Delta G^{0'}$ of the *hydrolytic reaction*, thus e.g. from creatine phosphate to ADP. At a high ATP concentration it is possible to *revert* the reaction by the transfer of phosphate groups from ATP to creatine. In muscles *creatine phosphate* may serve as an *energetic buffer*.

The reaction is catalyzed in both directions by the enzyme creatine kinase.

ENTHALPY CHANGE : ΔH
is a change in the heat content of the system
$\Delta H < 0$ (negative) - exothermic reaction (heat release)
$\Delta H > 0$ (positive) - endothermic reaction (heat absorption)

ΔH is heat of combustion:
e.g. for glucose :
$\Delta H = -2870\ kJ \cdot mol^{-1}$

3 STAGES OF CATABOLISM

I neutral fats, polysaccharides and proteins are *hydrolytically* broken down into their constituent *building blocks*

II through mutual metabolic *interconversions* their catabolites are converted into a *common intermediate*: acetyl-CoA

III acetyl- residue is *finally degraded* in the citric acid cycle to 2 CO_2 (via *decarboxylations*) and 4 pairs of electrons are taken up by 4 *dehydrogenases* from the intermediates of the cycle and transferred to the *respiratory chain* of the innner mitochondrial membrane, which *couples* their *terminal oxidation* to H_2O with *oxidative (aerobic) phosphorylation* producing ATP

BIOLOGICAL OXIDATIONS START AS DEHYDROGENATIONS

Coenzymes of dehydrogenases are:

1) *Pyridine* nucleotide NAD^+, which transfers 2 electrons in the reaction:

$NAD^+ + 2H \leftrightarrows NADH + H^+$

or

2) *Flavin* nucleotide - FMN or FAD, transferring 2 electrons in the reaction

$FMN + 2\,H \leftrightarrows FMNH_2$

or $FAD + 2\,H \leftrightarrows FADH_2$

In both cases the process starts as a *two-electron transfer*.

Terminal oxidation of 2 reducing equivalents in the inner mitochondrial membrane via transfer of 2 electrons to 1/2 O_2 is combined with pumping 10 H^+, if starting from NADH, but only 6 H^+, if starting from $FADH_2$, into the intermembrane space. (Flavin carriers have a more positive standard redox-potential and the difference in redox-potentials versus the oxygen half-cell [ΔE] is smaller.) For the synthesis of 1 ATP during *oxidative (aerobic) phosphorylation* (via membrane-bound *ATP/H^+ - synthase)* about 4 H^+ are returned into mitochondrial matrix.

CLASSIFICATION OF ENZYMES

1. OXIDOREDUCTASES

2. TRANSFERASES

3. HYDROLASES

4. LYASES

5. ISOMERASES

6. LIGASES

CLASSIFICATON OF ENZYMES REFLECTS THE BASIC TYPES OF METABOLIC REACTIONS

www.chem.qmul.ac.uk.iubmb/enzyme

Acetyl-CoA IS A COMMON INTERMEDIATE OF VARIOUS TYPES OF FOODSTUFFS

Activated acetyl group (as *acetyl-CoA*) is catabolized in the *citric acid cycle* in the mitochondrial matrix. It originates:
1) From *pyruvate* (as the product of *glycolysis* and/or of *transamination* from *glucogenic amino acids*) by *irreversible* process of *oxidative decarboxylation* in the multienzyme *pyruvate dehydrogenase complex.*
2) As the end-product of *β-oxidation of fatty acids* and from the carbon chain of *ketogenic amino acids.*

Coenzyme A functions as an *activated acyl carrier*.

CATABOLIC ROLE OF CITRIC ACID CYCLE

1) *Acetyl-CoA* is a *common intermediate* in the *catabolism* of carbohydrates, lipids and proteins.
2) *Citric acid cycle* in the mitochondrial matrix operates as a *common „metabolic mill“* and supplies *reducing equivalents* (electrons, hydrogen atoms) to the respiratory chain of the inner mitochondrial membrane.
3) *Respiratory chain* transfers the reducing equivalents up to *molecular oxygen* , whereby its *electron carriers* (complexes I, III and IV) function as *proton pumps*, extruding protons from the matrix into the intermembrane space.
4) *Protons flow back* down their concentration slope driving the enzyme *ATP/H^+-synthase*, which forms ATP by the mechanism of *oxidative (aerobic) phosphorylation.*

CATALYTIC FUNCTION OF A METABOLIC CYCLE

The significance of *Krebs* citric acid cycle (and of any metabolic cycle, e.g. ornithin [urea] cycle, also discovered by Krebs) is given by the fact that *each member of the cycle acts as a catalyst.*

Oxaloacetate is consumed in the condensation reaction with acetyl-CoA and is regenerated. Theoretically a single molecule of oxaloacetate could - during a continuous supply of acetyl-CoA - serve an infinite number of cycles, *unless oxaloacetate were consumed in other reactions.* This holds also for other members of the cycle.

ENERGETIC BALANCE OF CITRIC ACID CYCLE

after combustion of 1 acetyl group (1 turn of the cycle)

isocitrate dehydrogenase	NAD	2.5 ATP
2-oxoglutarate dehydrogenase	NAD	2.5 ATP
succinate thiokinase	(substrate phosphorylation)	1 GTP (ATP)
succinate dehydrogenase	FAD	1.5 ATP
malate dehydrogenase	NAD	2.5 ATP

SUM ~ 10 ATP

ENERGETIC CONTROL OF CITRIC ACID CYCLE

ATP and NADH inhibit:

- pyruvate dehydrogenase
- key enzymes of citric acid cycle:
    - isocitrate dehydrogenase
    - 2-oxoglutarate dehydrogenase

CITRIC ACID CYCLE IS THE SOURCE OF PRECURSORS FOR BIOSYNTHETIC (ANABOLIC) REACTIONS (AMPHIBOLIC NATURE OF THE CYCLE)

citrate: fatty acids, sterols
2-oxoglutarate: glutamate, amino acids, purines
succinyl-CoA: porphyrins, heme
oxaloacetate: aspartate, amino acids, purines, pyrimidines

***Oxaloacetate* is a precursor of *phosphoenolpyruvate* in gluconeogenesis. With the help of GTP the reaction is catalyzed by the enzyme phosphoenolpyruvate carboxykinase.**

ANAPLEROTIC REACTIONS OF CITRIC ACID CYCLE

Metabolic intermediates of the cycle are *consumed* in accessory, chiefly *anabolic reactions*, and therefore it is necessary *to recover* them in *replenishing (anaplerotic) reactions*.

Oxaloacetate is replenished by the *carboxylation of pyruvate* with the help of ATP by the biotin-dependent enzyme **pyruvate carboxylase.**

In citric acid cycle, as in any metabolic cycle, the replenishment of any member of the cycle by an supplementary reaction has an anaplerotic character.