ENZYMES

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ENZYMES AS HOMOGENEOUS BIOKATALYSTS

1. Protein nature (+ some are RNAs – ribozymes)
2. Higher efficiency (factor at least 10^6 x)
3. Specificity - substrate type
   - reaction type
4. Regulation - genomic (induction, repression)
   - at the level of enzyme (allosteric, covalent)
   - proteolytic (precursors – zymogens)
5. Compartmentation
6. Decreasing activation energy, do not change equilibrium constant
   - approximation of reactants
   - stabilization of transition state – activated complex

EFFICIENCY OF ENZYME CATALYSIS (EXAMPLES)

decomposition of 2 moles H_2O_2 to 2 H_2O and O_2	**E_a**
non-catalyzed reaction	75 kJ . mol^{-1}
catalysis by colloidal platinum	45 kJ . mol^{-1}
catalysis by catalase	8 kJ . mol^{-1}

Acceleration of a reaction 10-fold at a given temperature lowers the activation energy by a value: - *R.T.* ln10, ie. for 37 °C (310.15 K) = - 5.9 kJ . mol^{-1} (log 10)

For a non-catalyzed reaction it can be roughly assumed that increase in temperature by 10 °C accelerates the reaction 2- to 4- fold (temperature coefficient Q_{10} : van´t Hoff rule)

TURNOVER NUMBERS (maximum molar activity) of some enzymes (k_{cat}) (mol . mol^{-1}) s^{-1}

Carbonic anhydrase	**600,000**
Acetylcholinesterase	**25,000**
Lactate dehydrogenase	**1000**
Chymotrypsin	**100**
DNA - polymerase I	**15**
Lysozyme	**0.5**

For most physiological substrates the range is 1 - 10^4 molecules per second transformed by 1 molecule of enzyme under optimum conditions - full saturation of enzyme with substrate.

ACTIVATION ENERGY E_a (ΔG^*)

is the minimum amount of energy required to initiate a chemical reaction. Its value is always positive (endergonic reaction).

Collision theory claims that a critical *frequency of efficient collisions* of reacting molecules is needed to overcome the energetic barriers of electronic shells of the molecules. The proportion of *effective* collisions versus the *ineffective* ones is very small (e.g. $1:10^{14}$) and depends on temperature (kinetic energy od the colliding molecules) and the concentration of reactants.

Transition state theory assumes that a critical *concentration* of *activated complex (transition state* containing more energy) is needed to initiate the reaction.
ΔG^* is the difference in free enthalpy (energy) of the activated complex and the substrate and is related to the equilibrium constant of the formation and disappearance of the activated complex. **Enzymes** facilitate the formation of the activated complex.

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

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

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, after reaction of 1 mole of a substance

(**´ =** pH 7). **Free** energy (free enthalpy) is such, which may **perform work under isothermic conditions .** However**,** energetic balance of an 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 **$\Delta G'$** .

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

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

At equilibrium the term **$\Delta G'$** equals **zero.**

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

At 37 °C and pH 7 it reads:

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

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

ACTIVE SITE OF AN ENZYME

is a relatively *small* three-dimensional *cleft* inside the enzyme molecule or at its surface, often *hydrophobic*, which enables the binding of substrate(s) and/or cofactor (coenzyme) by weaker, *transitional*, *noncovalent* interactions :

- hydrogen bonds
- electrostatic interactions
- hydrophobic interactions
- van der Waals forces

It contains *side chains* and *binding groups* coming from *different parts* of amino acid sequence of *polypeptide chain*, which represent *contact*, *orienting* and *catalytic* residues (often polar) and form a *biospecific conformation*. In this way a *reversible* and *transitory* enzyme-substrate complex (ES) is formed. Biocatalysis may have a character of *general acid-base catalysis* (proton donors and acceptors other than water), *metal-ion catalysis* (Lewis acids), sometimes *covalent catalysis*. During the formation of the activated complex following entities may *temporarily* take part: a covalently bound *proton*, a covalently bound *metallic atom* or a *residue* representing a *Brönsted base* (enabling a *nucleophilic* attack); or the *intermediate product* may be temporarily bound covalently.

SMALL NON-PROTEIN COMPONENTS OF ENZYMES (COFACTORS)

- divalent cations : Zn^{2+}, Mg^{2+}, Cu^{2+}, Mn^{2+}, Ca^{2+}
- coenzymes (relationship to vitamins) - bound noncovalently
- prosthetic group (heme) - bound covalently

necessary for the mechanism of action of some enzymes

COENZYMES AND VITAMINS

Vitamin	Coenzyme	Function
B_3 Niacin	NAD, NADP	Transfer of 2 e^- + H^+
B_2 Riboflavin	FAD, FMN	Transfer of 2 H
B_1 Thiamin	TDP (TPP)	Oxidative decarboxylation
B_6 Pyridoxal(-ine,-amine)	PLP	Transamination, decarboxylation of AA
H Biotin	Biotin coenzyme	Carboxylation
Folic acid	THF (contains PABA)	Transfer of 1 C - residues
B_{12}	Cobamide	Transfer of 1 C - residues
B_5 Pantothenic acid	Coenzyme A	Acyl - transfer

CLASSIFICATION OF ENZYMES (EC SYSTEM)

6 classes following the type of reaction, subclasses
name: substrate - type of reaction - ase
additional information (parentheses)
systematic code number EC (4 digits)
(recommended trivial names)
e.g.: EC 2.7.1.1.
transferase - phosphate transfer - alcohol acceptor - enzyme
ATP: D-hexose-6-phosphotrasferase (hexokinase)
G + ATP <=> G-6-P + ADP

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

CLASSIFICATION OF ENZYMES

1. OXIDOREDUCTASES
2. TRANSFERASES
3. HYDROLASES
4. LYASES
5. ISOMERASES
6. LIGASES

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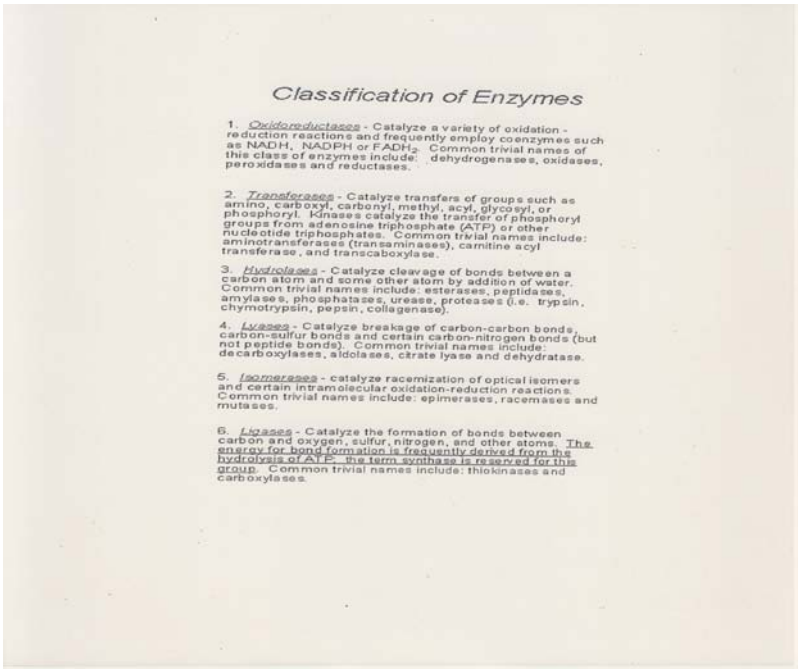

Classification of Enzymes

1. *Oxidoreductases* - Catalyze a variety of oxidation - reduction reactions and frequently employ coenzymes such as NADH, NADPH or $FADH_2$. Common trivial names of this class of enzymes include: dehydrogenases, oxidases, peroxidases and reductases.

2. *Transferases* - Catalyze transfers of groups such as amino, carboxyl, carbonyl, methyl, acyl, glycosyl, or phosphoryl. Kinases catalyze the transfer of phosphoryl groups from adenosine triphosphate (ATP) or other nucleotide triphosphates. Common trivial names include: aminotransferases (transaminases), carnitine acyl transferase, and transcaboxylase.

3. *Hydrolases* - Catalyze cleavage of bonds between a carbon atom and some other atom by addition of water. Common trivial names include: esterases, peptidases, amylases, phosphatases, urease, proteases (i.e. trypsin, chymotrypsin, pepsin, collagenase).

4. *Lyases* - Catalyze breakage of carbon-carbon bonds, carbon-sulfur bonds and certain carbon-nitrogen bonds (but not peptide bonds). Common trivial names include: decarboxylases, aldolases, citrate lyase and dehydratase.

5. *Isomerases* - catalyze racemization of optical isomers and certain intramolecular oxidation-reduction reactions. Common trivial names include: epimerases, racemases and mutases.

6. *Ligases* - Catalyze the formation of bonds between carbon and oxygen, sulfur, nitrogen, and other atoms. The energy for bond formation is frequently derived from the hydrolysis of ATP. The term synthase is reserved for this group. Common trivial names include: thiokinases and carboxylases.

CLASSIFICATION OF PROTEASES (PEPTIDASES)

3.4.21 Serine endopeptidases
- chymotrypsin, trypsin, thrombin, plasmin, enterokinase

3.4.22 Cysteine endopeptidases
- cathepsin B, papain, bromelain, caspases

3.4.23 Aspartic endopetidases
- pepsin

3.4.24 Metalloendopeptidase
- procollagen-N-proteinase

3.4.17.1 Carboxypeptidase A (contains Zn)

UNITS EXPRESSING ENZYME ACTIVITY

katal (abbrev.: kat) : amount of enzyme activity catalyzing conversion of 1 mole of substrate into 1 mole of product per second

10^{-6} kat = μkat 10^{-9} kat = nkat

international unit IU: amount of enzyme activity catalyzing conversion of 1 μmole of substrate into 1 μmole of product per minute:10^{-3} IU = mU

Conversion: 1 IU = 16.67 nkat

BIOLOGICAL AND CLINICAL SIGNIFICANCE OF ENZYMES

Enzymes are tools of gene expression. They enable turnover of substances (metabolism) and energy transformation in the organism.

In the medicine:

1. Inborn and hereditary enzyme defect causes an inborn error of metabolism.

2. Cell and tissue damage during a disease is reflected by an increased activity of the released enzyme in plasma.

3. Enzymes are used in substitution therapy.

4. They may serve as diagnostic tools.

5. Enzyme are used as tools for industrial drug production.

SEPARATION AND PURIFICATION OF ENZYMES

1. Based on different solubility: fractional precipitation, crystallization
2. Based on different pI: electrophoresis, ion exchange chromatography
3. Based on different M_r: gel permeation chromatography, ultrafiltration, ultracentrifugation
4. Biospecific separation: affinity chromatography

ISOENZYMES (ISOZYMES)

are multiple forms of enzymes ocurring in the same species and catalyzing the same reaction. They have different sequence of amino acids in their primary structure being coded for by distinct genes, which originated by gene duplication or divergence. Example: lactate dehydrogenase. They differ e.g. in electrophoretic mobility, pI, pH-optimum, substrate specificity (K_m), sensitivity towards inhibitors. Isoenzymes are expression of developmental, organ and cell differentiation and specialization. Some are employed as significant diagnostic tools.

FACTORS INFLUENCING ENZYME ACTIVITY

1. substrate concentration (K_m, V, K_{cat})
2. temperature (optimum)
3. pH (optimum)
4. ionic strength
5. activators and inhibitors