

3rd lecture: ENZYMES



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ENZYMES

A many proteins are known with different biological functions:

- Regulator proteins
- Transport proteins
- Protecting proteins
- Toxins
- Reserve proteins
- Contractile proteins
- Structural proteins

ENZYMES - catalysts of reactions

ε ν ζ υ μ η = "in yeast" (greek) 1878 Kühne



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THERMODYNAMICS OF CATALYSIS

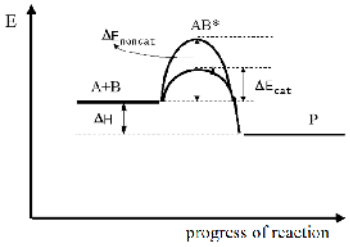

1930- years: Eyring:

During the reaction a higher energy transition complex is formed - activation energy (E*) is needed:

$$k_r = \frac{kT}{h} e^{-\frac{\Delta S^\ddagger}{R}} \cdot e^{-\frac{\Delta H^\ddagger}{RT}} \approx \text{const} \cdot e^{-\frac{\Delta E^\ddagger}{RT}}$$

k_r - reaction rate constant
 T - absolute temperature (Kelvin)
 k - Boltzmann constant (1.37.10⁻²³ J°K)
 h - Planck constant (6,62.10⁻³⁴ Js)

This energy is reduced by catalysts – the reaction rate is higher but the chemical equilibrium is not affected.

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Comparison of chemical and enzymatic catalysis

Reaction	Catalyst	Activation energy kJ/mol	k_{rel} 25 °C
$H_2O_2 \rightarrow H_2O + 1/2O_2$	-	75	1
	I^-	56,5	$2,1 \cdot 10^3$
	catalase	26,8	$3,5 \cdot 10^8$
Casein + nH_2O (n+1) peptide	H^+	86	1
	trypsin	50	$2,1 \cdot 10^6$
Sucrose + H_2O glucose+fructose	H^+	107	1
	invertase	46	$5,6 \cdot 10^{10}$
Linoleic acid + O_2 linolene peroxide	-	150-270	1
	Cu^{2+}	30-50	$\sim 10^2$
	lipoxygenase	16,7	$\sim 10^7$

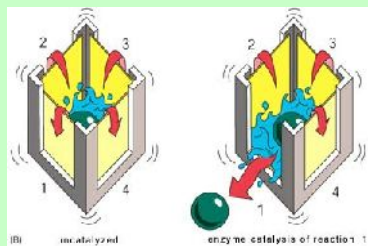
Catalysis

General cases of the enzymatic catalysis
(taken from general chemistry):

1. acid-base catalysis
2. covalent catalysis
3. metal ion catalysis

ENZYMES

In a cell the organic compounds may react on many different way – but these reactions are very slow because of the activation energy barrier. The enzymes open a certain reaction route.



Enzyme-substrate complex

A higher energy transition complex is formed:



The substrate attached to the substrate binding site, that is only a small portion of the surface of the enzyme molecule (sack/pocket).

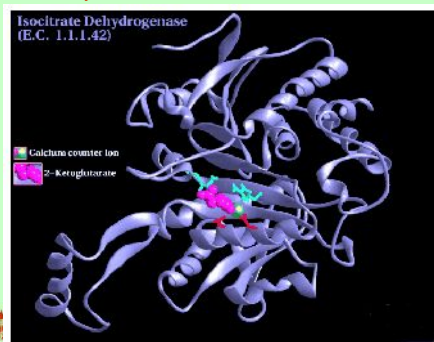
Other domains on the surface:

- Catalytic domain = **ACTIVE CENTER** – the site for chemical reaction
- Sites for modulators (inhibitors, activators, S, P, metal ions)
- Sites for covalent modification of enzyme (phosphorylation, glycosylation, proteolysis)



Substrate binding site

The substrate binding site is only a small spot/pocket on the surface of enzyme molecule



Enzyme-substrate interactions

... between the molecular surfaces:

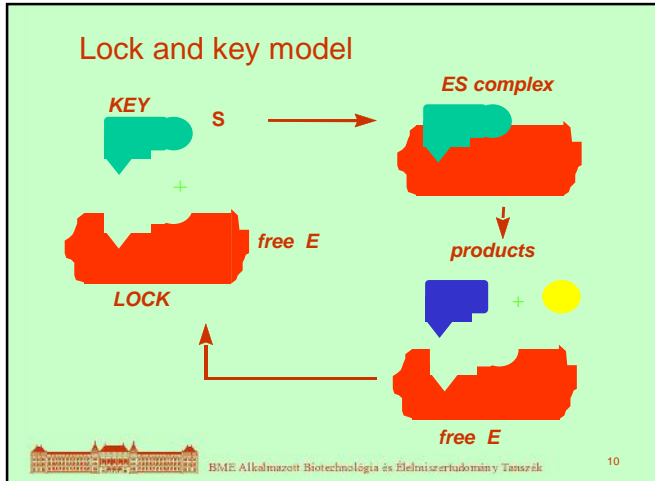
Secondary (noncovalent) interactions:

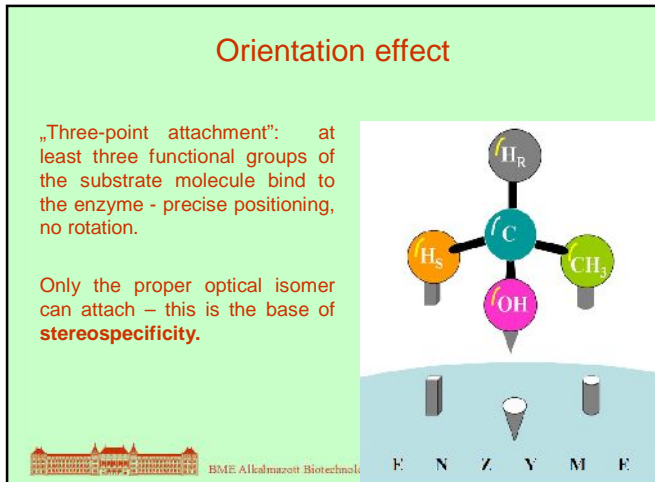
- electrostatic
- Van der Waals and
- hydrophobic interactions

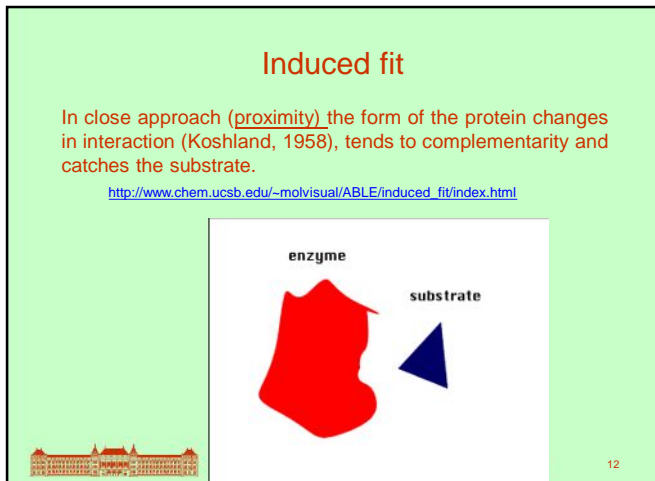
Effects in enzyme-catalysis:

- lock and key model
- proximity effect
- orientation effect
- induced fit (Koshland-conformation change)





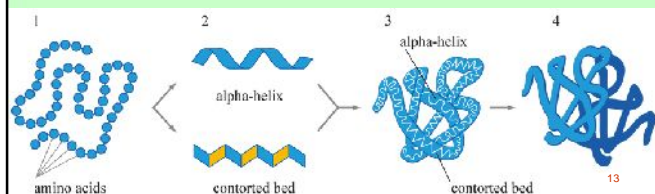




How is the proper surface formed?

The folded peptide chains form the three dimensional structure of protein (tertiary, quaternary structure). The side chains of amino acids can be:

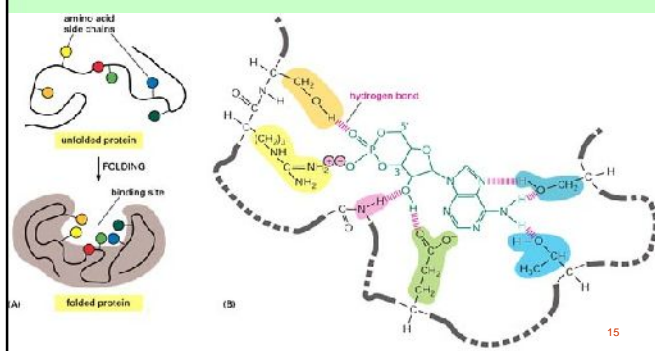
- apolar (alkyl groups)
- polar (-OH, -SH groups)
- ionic (-NH₂, -COOH groups)



Reactive side chains

Acidic: -COOH: Asp, Glu Basic: -NH₂: Lys, Arg
 terminal -COOH and -NH₂
 Amide: -CO-NH₂: Asn, Gln
 Polar: -OH: Ser, Thr -SH: Cys, -S-CH₃: Met
 Imidazole: His Guanidine: Arg
 H-bonds: C=O H-O- C=O H-NH-

Conformation of active center




Enzyme catalysed reactions

Only thermodynamically possible reactions can be catalysed
 $\Delta G < 0$

All enzyme catalysed reactions are reversible, tends to an equilibrium. but: the equilibrium can be shifted, e.g.. with product removal.

Proteins are denaturable: t, pH, ionic strength (salting out), organic solvents

Specificity: substrate-specificity
 group-specificity
 stereo-specificity
 region-specificity



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Pros for enzyme catalysed reactions

Higher reaction rate: even 10^6 - 10^{12} x faster

Mild reaction condition (temperature, pressure, pH)

Sophisticated selectivity, better than in organic chemistry

Easy control



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Necessary reaction partners

HOLOENZYME

┌───────────┴───────────┐

APOENZYME + COFACTOR

┌───────────┴───────────┐


METAL ION

Mg, Ca, Zn,
Fe, Cu, Mo

COENZYME

Prosthetic group
stable covalent bond
FAD(H₂), Pyridoxal-P(B₆)

Cosubstrate
Stoichiometric use,
must be regenerated
NAD(H), ATP



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Nomenclature of enzymes

- To substrate: $\text{urea} + \text{water} \rightleftharpoons \text{CO}_2 + 2\text{NH}_3$
 urea → **urease** S-name + ase
- To substrate and reaction: $\text{EtOH} \rightarrow \text{AcO} \rightarrow \text{AcOH}$
 EtOH → **alcohol-dehydrogenase** S-name + reaction name + ase
- Trivial names: pepsin, trypsin, rennin – all peptidases + -in
- IUB, IUPAC, IUBMB 1964,1972,1978 Enzyme Commission: systematical nomenclature

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Nomenclature of enzymes

catalogue number
cosubstrate

E.C.1.1.1.49. D-glucose-6P: NADP 1-oxydoreductase

substrate
target on the 1st C-atom
the reaction

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Group	Reaction catalyzed	Typical reaction	Enzyme example(s) with trivial name
EC 1 Oxidoreductases	To catalyze oxidation/reduction reactions; transfer of H and O atoms or electrons from one substance to another	$\text{AH} + \text{B} \rightarrow \text{A} + \text{BH}$ (reduced) $\text{A} + \text{O} \rightarrow \text{AO}$ (oxidized)	Dehydrogenase, oxidase
EC 2 Transferases	Transfer of a functional group from one substance to another. The group may be methyl-, acyl-, amino- or phosphate group	$\text{AB} + \text{C} \rightarrow \text{A} + \text{BC}$	Transaminase, kinase
EC 3 Hydrolases	Formation of two products from a substrate by hydrolysis	$\text{AB} + \text{H}_2\text{O} \rightarrow \text{AOH} + \text{BH}$	Lipase, amylase, peptidase
EC 4 Lyases	Non-hydrolytic addition or removal of groups from substrates. C-C, C-N, C-O or C-S bonds may be cleaved	$\text{RCO}_2\text{COOH} \rightarrow \text{RCOH} + \text{CO}_2$ or $[\text{X-A-B-Y}] \rightarrow [\text{A=B} + \text{X-Y}]$	Decarboxylase
EC 5 Isomerases	Intramolecule rearrangement, i.e. isomerization changes within a single molecule	$\text{AB} \rightarrow \text{BA}$	Isomerase, mutase
EC 6 Ligases	Join together two molecules by synthesis of new C-O, C-S, C-N or C-C bonds with simultaneous breakdown of ATP	$\text{X} + \text{Y} + \text{ATP} \rightarrow \text{XY} + \text{ADP} + \text{Pi}$	Synthetase

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