

3rd lecture: ENZYMES



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	$\sim 10^7$



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

$\epsilon \nu \zeta \upsilon \mu \eta$ = "in yeast" (greek) 1878 Kühne



Catalysis

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

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



THERMODYNAMICS OF CATALYSIS

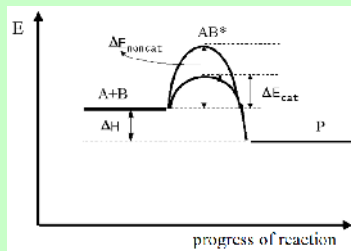
1930- years: Eyring:

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

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

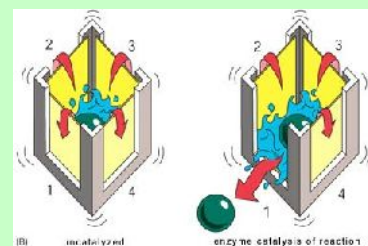
- k_r - reaction rate constant
- T - absolute temperature (Kelvin)
- k - Boltzmann constant ($1,37 \cdot 10^{-23} J/K$)
- h - Planck constant ($6,62 \cdot 10^{-34} Js$)

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



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:
 $E + S \rightleftharpoons ES^* \rightarrow E + P$

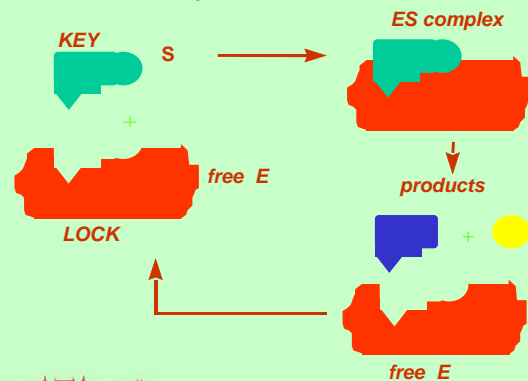
The substrate attached to the substrate binding site, that is only a small portion of the surface of the enzyme molecule (sack/pocket).

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)

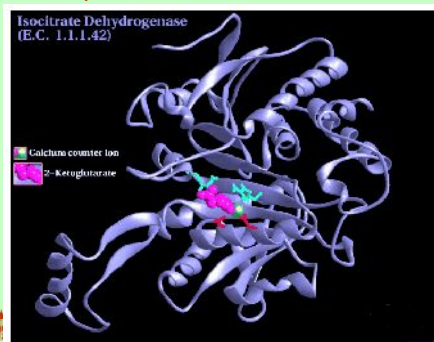


Lock and key model



Substrate binding site

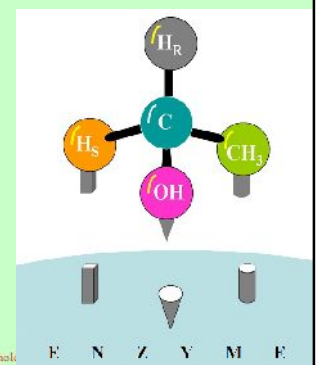
The substrate binding site is only a small spot/pocket on the surface of enzyme molecule



Orientation effect

„Three-point attachment”: at least three functional groups of the substrate molecule bind to the enzyme - precise positioning, no rotation.

Only the proper optical isomer can attach – this is the base of **stereospecificity**.



Enzyme-substrate interactions

... between the molecular surfaces:
 Secondary (noncovalent) interactions:

- electrostatic
- Van der Waals and
- hydrophobic interactions

Effects in enzyme-catalysis:

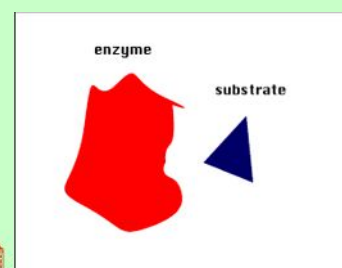
- lock and key model
- proximity effect
- orientation effect
- induced fit (Koshland-conformation change)



Induced fit

In close approach (proximity) the form of the protein changes in interaction (Koshland, 1958), tends to complementarity and catches the substrate.

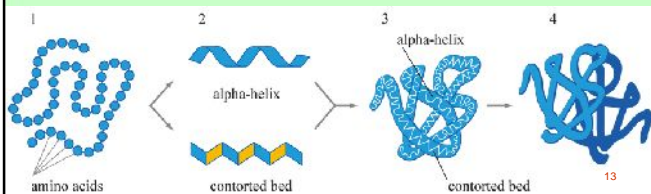
http://www.chem.ucsb.edu/~molvisual/ABLE/induced_fit/index.html



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)



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



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-



Pros for enzyme catalysed reactions

Higher reaction rate: even 10⁶-10¹² x faster

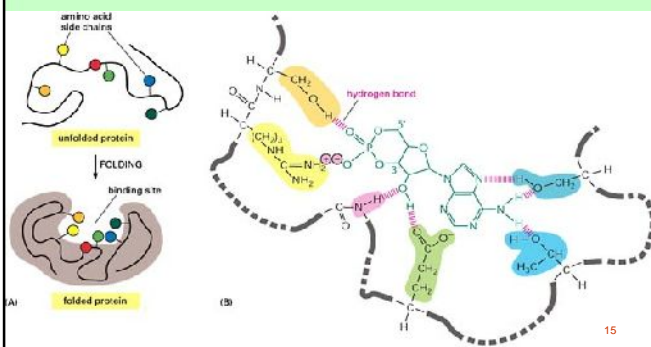
Mild reaction condition (temperature, pressure, pH)

Sophisticated selectivity, better than in organic chemistry

Easy control



Conformation of active center



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



Nomenclature of enzymes

1. To substrate: $\text{urea} + \text{water} \rightleftharpoons \text{CO}_2 + 2\text{NH}_3$
 urea → **urease** **S-name + ase**
2. To substrate and reaction: $\text{EtOH} \rightarrow \text{AcO} \rightarrow \text{AcOH}$
 EtOH → **alcohol-dehydrogenase** **S-name + reaction name + ase**
3. Trivial names: pepsin, trypsin, rennin – all peptidases **+ -in**
4. 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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