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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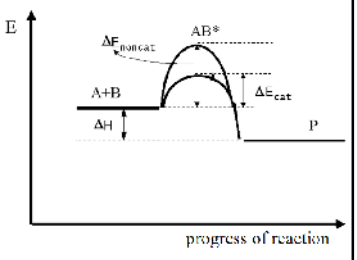
THERMODYNAMICS OF CATALYSIS

1930- years: Eyring:

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

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



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
$H_2O_2 \rightarrow H_2O + 1/2O_2$	- I^{-1} catalase	75 56,5 26,8	1 $2{,}1.10^3$ $3{,}5.10^8$
Casein + nH_2O →(n+1) peptide	H^+ trypsin	86 50	1 $2{,}1.10^6$
Sucrose + H_2O → glucose+fructose	H^+ invertase	107 46	1 $5{,}6.10^{10}$
Linoleic acid + O_2 → linolene peroxide	- Cu^{2+} lipoxygenase	150-270 30-50 16,7	1 $\sim 10^2$ $\sim 10^7$

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Catalysis

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

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

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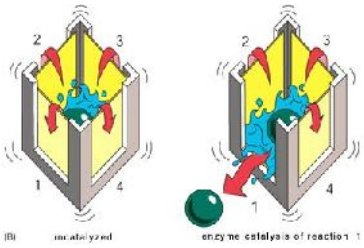
ENZYMES

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.



(B) enzyme catalysis of reaction 1

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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)

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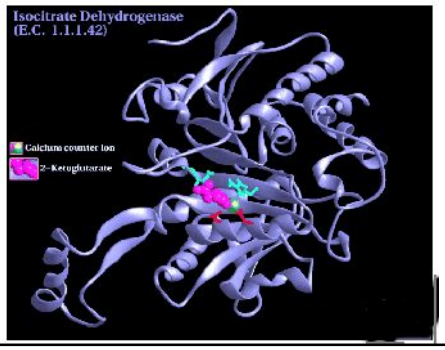
Substrate binding site

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



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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)

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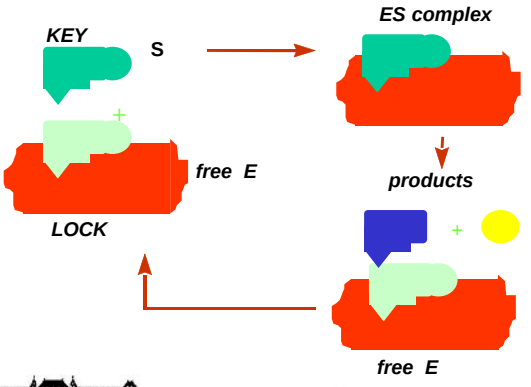
Lock and key model



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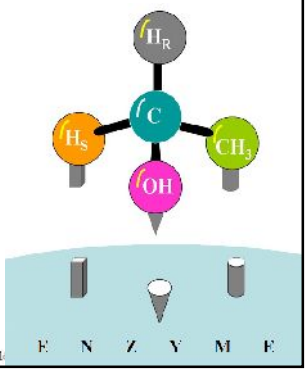
Orientation effect

„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.**



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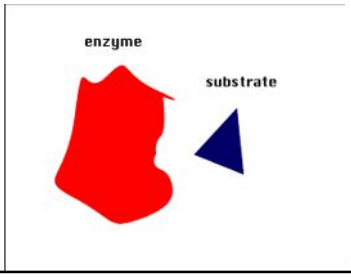
Induced fit

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



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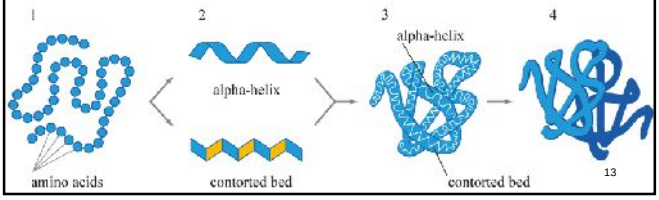
How is the proper surface formed?

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 (-NH_2, -COOH groups)



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Reactive side chains

Acidic: –COOH: Asp, Glu Basic: -NH_2: Lys, Arg
terminal –COOH and -NH_2
Amide: –CO-NH_2: Asn, Gln

Polar: -OH: Ser, Thr -SH: Cys, -S-CH_3: Met

Imidazole: His Guanidine: Arg

H-bonds: C=O …… H-O- C=O …… H-NH-

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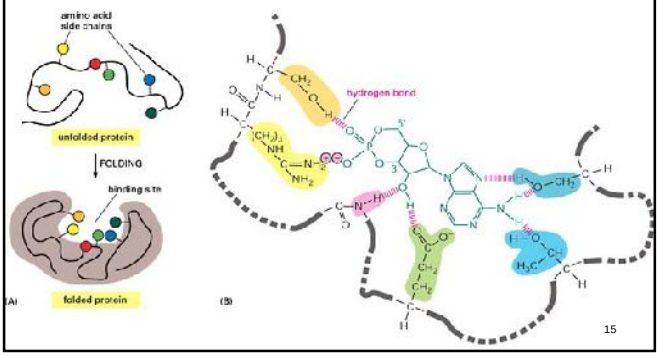
Conformation of active center



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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

Specifity: substrate-specifity
group-specifity
stereo-specifity
region-specifity

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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

Prostetic group
stable covalent bond
FAD(H_2), Pyridoxal-P(B_6)

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

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Nomenclature of enzymes

1. To substrate: urea + water ⇌ CO_2 + $2NH_3$

urease

S-name + ase

2. To substrate and reaction: EtOH → AcO → AcOH

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

the reaction

substrate

target on the 1st C-atom

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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	AH + B → A + BH (reduced) A + O → 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	AB + C → A + BC	Transaminase, kinase
EC 3 Hydrolases	Formation of two products from a substrate by hydrolysis	AB + H_2O → AOH + 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	RCOCOOH → RCOH + CO_2 or [X-A-B-Y] → [A=B + X-Y]	Decarboxylase
EC 5 Isomerases	Intramolecule rearrangement, i.e. isomerization changes within a single molecule	AB → 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	X + Y+ ATP → XY + ADP + Pi	Synthetase

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