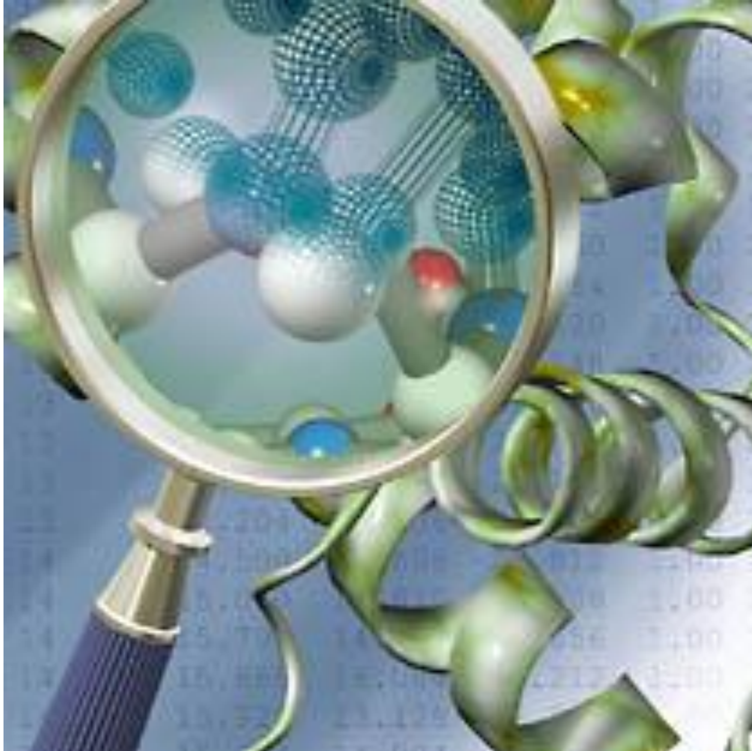


Enzymes

BASICS of enzymology - Enzyme Kinetics

Biochemistry-3

Two basic conditions of life

1. Living organisms must be capable of self-replication

2. Organisms must be capable of catalyzing chemical reactions efficiently and selectively

-Enzymes are biological catalysts systems enabling chemical transformations. They also allow the transformation of one form of energy to another.

-For enzymes characteristic catalytic power and specificity.

- The catalytic power of the enzyme is defined as the ratio of the rate of reaction catalyzed by the enzyme reaction rate and uncatalyzed.

- Catalysis takes place in the enzyme molecule called **active site**.

- A substance which catalyses the conversion is called a **substrate**.

- Nearly all known enzymes are proteins (RNA are probably the earliest catalysts - ribozymes).

General properties of enzymes

- proteins
- biocatalysts

  specific (due to the substrate effect)

  highly active

  operate under mild conditions

  may be regulated

  in vitro - sensitive to external conditions

Enzymes are different proteins

-simple proteins

- with covalently bound **prostetic group**

- metalloenzymes

- oligomeric, multienzyme complexes

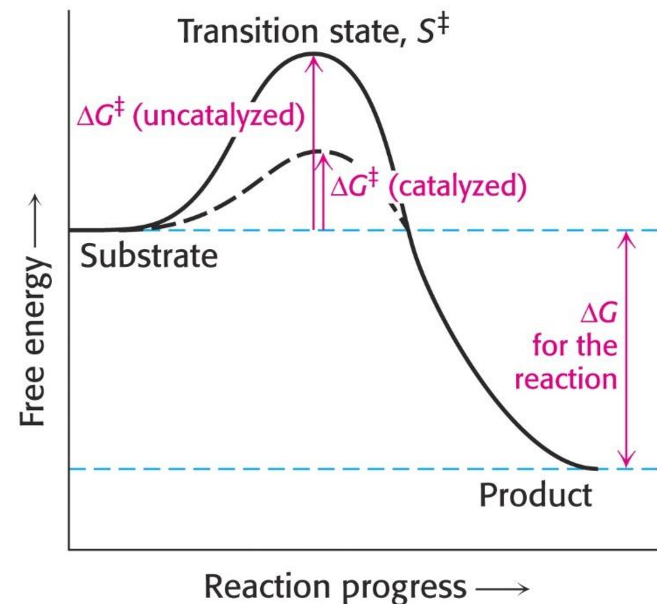
- associated with membranes

- differently distributed in body/cell

- forms isoforms

Enzymes are highly efficient catalysts

- Enzymes are highly efficient catalysts
- reduce the activation energy ⇒ accelerate reactions
  -efficiency by several orders of magnitude higher than that of other catalysts
  - reaction with the enzyme is about 10 6-10 14 faster than without enzyme
  do not affect the equilibrium constant K
  - relatively low stable



If the reaction biol. systems were catalyzed by enzymes, they would be so slow that it could not ensure the existence of living matter

Enzymes work under mild conditions

- atmospheric pressure
- narrow temperature range - about 37 ° C.
-

above 50 ° C usually denatured

- ⇒ narrow pH range
- pH optimum

There is a possibility of regulation

The enzyme activity

- -activators
- -inhibitors
- - covalent modification (phosphorylation)

The amount of enzyme

- -regulation of protein synthesis
- - enzyme proteolysis
- - some hormones - inducers × repressors

The specificity of enzymes is double

Consecutive, effects of the possible reactions

they catalyze only a single substrate

Substrate specificity

-possible substrates for a reaction can choose one (or a single group of substrates)

-often stereospecific

Enzymes are stereospecific catalysts

- There are two types of transformations:

  conversion of an **achiral substrate** to a **chiral product** (= single enantiomer), for example. pyruvate → L-lactate

  - transformation of a **chiral substrate** (only one enantiomer) of **the product** (also important for pharmacology)

L-alanin → pyruvate (D-alanin do not interact)

D-glucose → → pyruvate (L-glucose do not interact)

chiral signal molecule → complex with receptor → biological answer

chiral drug$_{\text{(ant)agonista}}$ → complex with receptor → farmacological answer

Example: hydrogenation of pyruvate

in vitro

Non-enzymatic

created racemate

(D, L-lactate)

in vivo

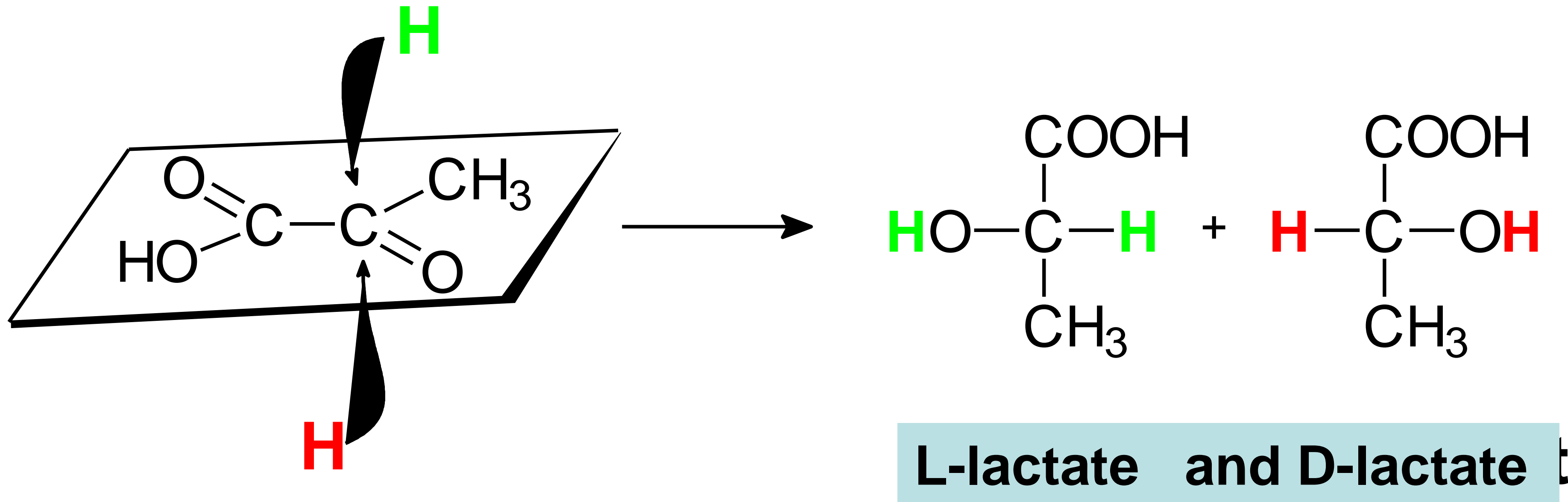
enzyme

created only one

enantiomer (L-lactate)

Hydrogenation of pyruvate *in vitro*

adition of hydrogen to planar substrate from both sides



There are statistically the same probability approach reagent from both sides of a planar substrate - therefore arises racemate

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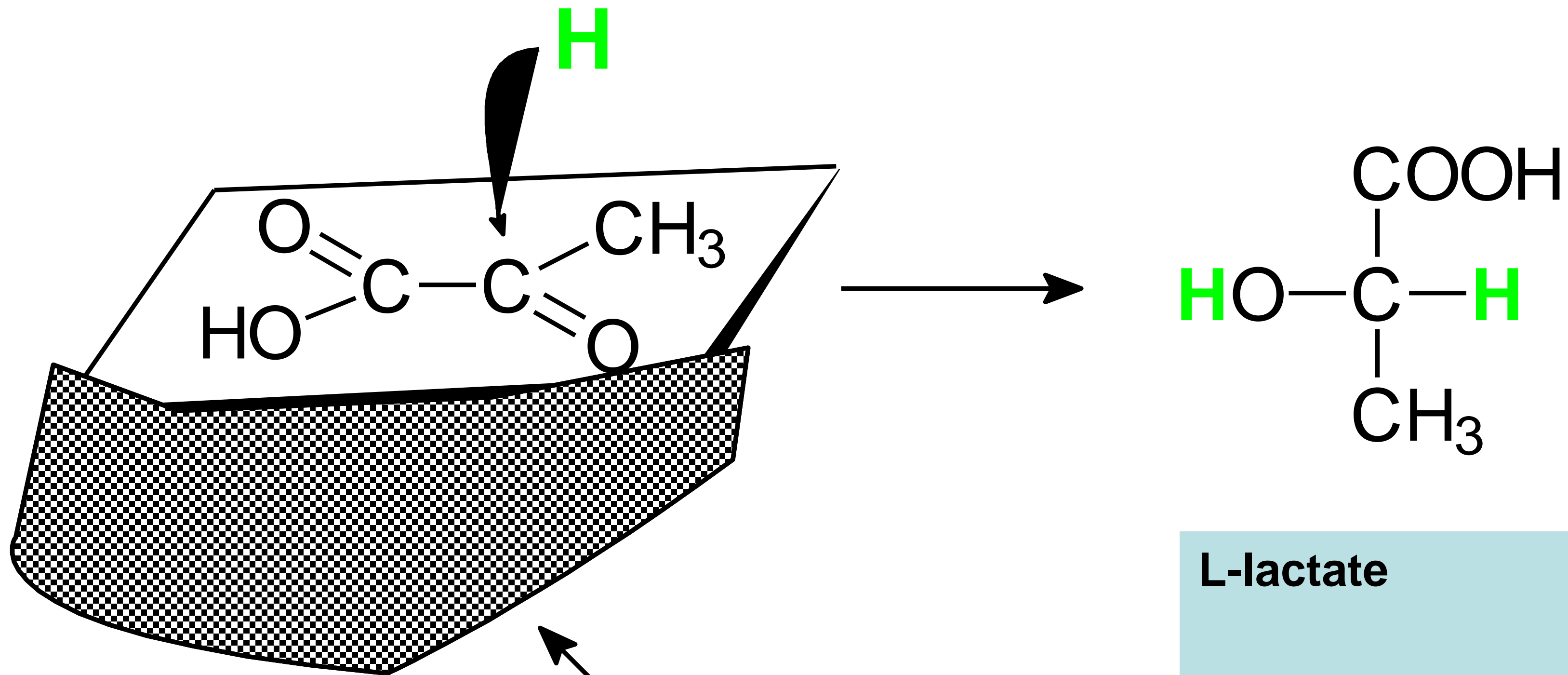
Hydrogenation of pyruvate in vivo (anaerobic glycolysis)

addition of hydrogen to the planar substrate from one side only



L-lactate

Only 1 stereoisomere

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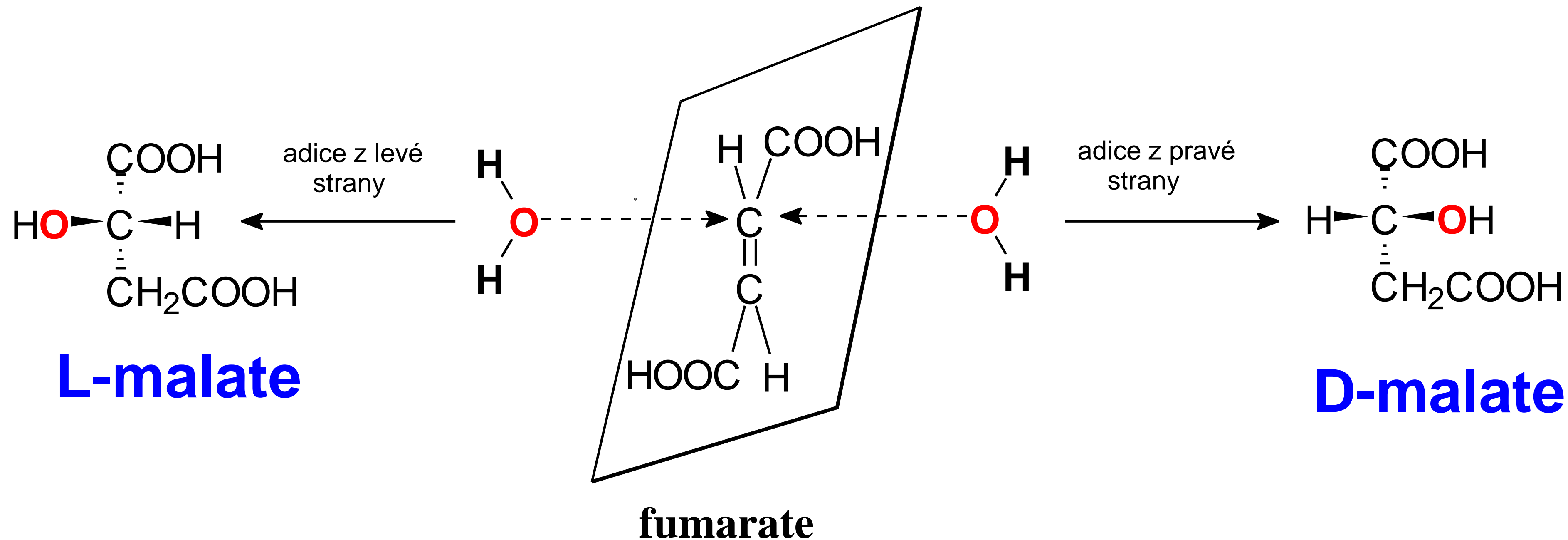
Example: Non-enzymatic hydration of fumarate



in vitro formed racemic D, L-malate

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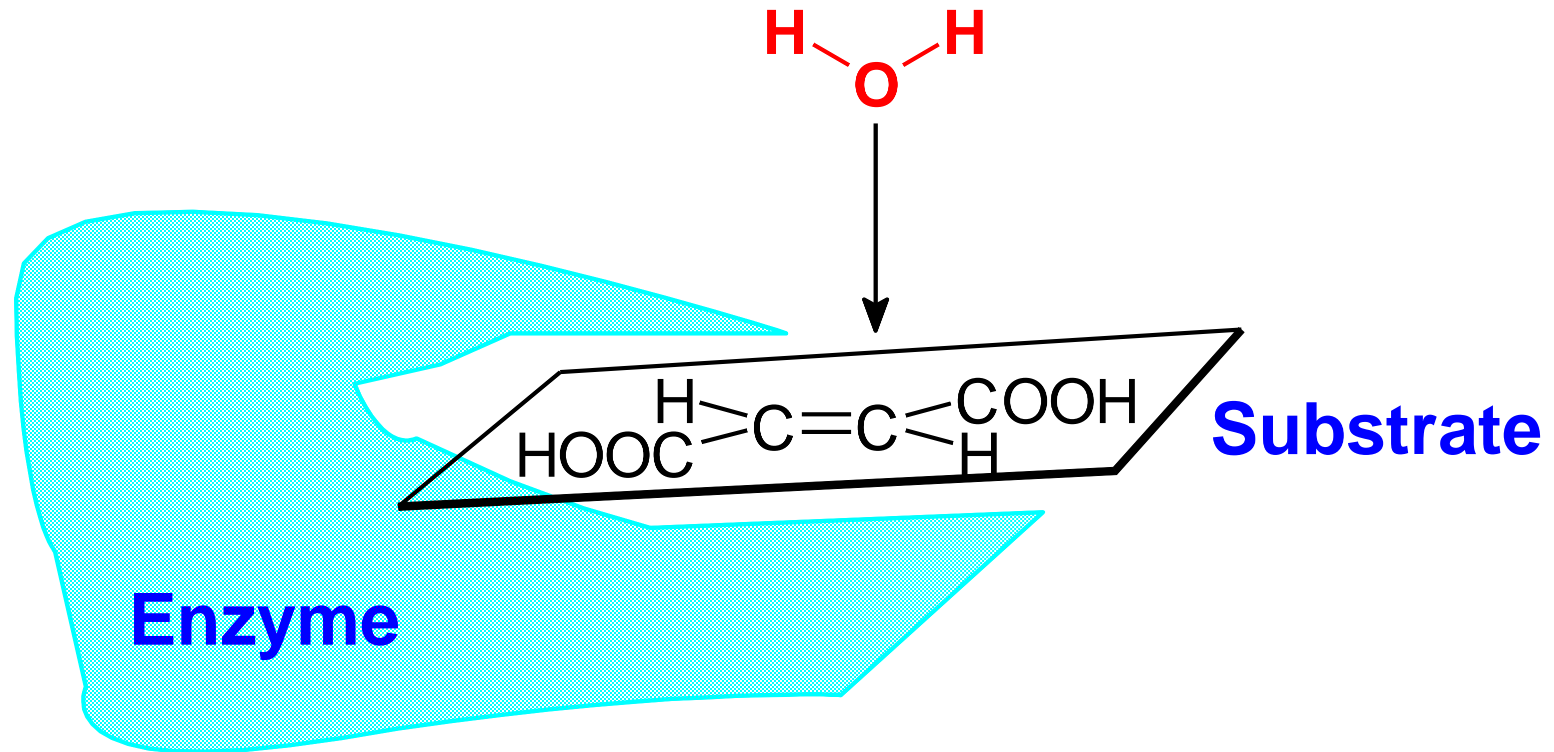
Enzyme hydration of fumarate (CC)



occurs in vivo, only one enantiomer (L-malate)

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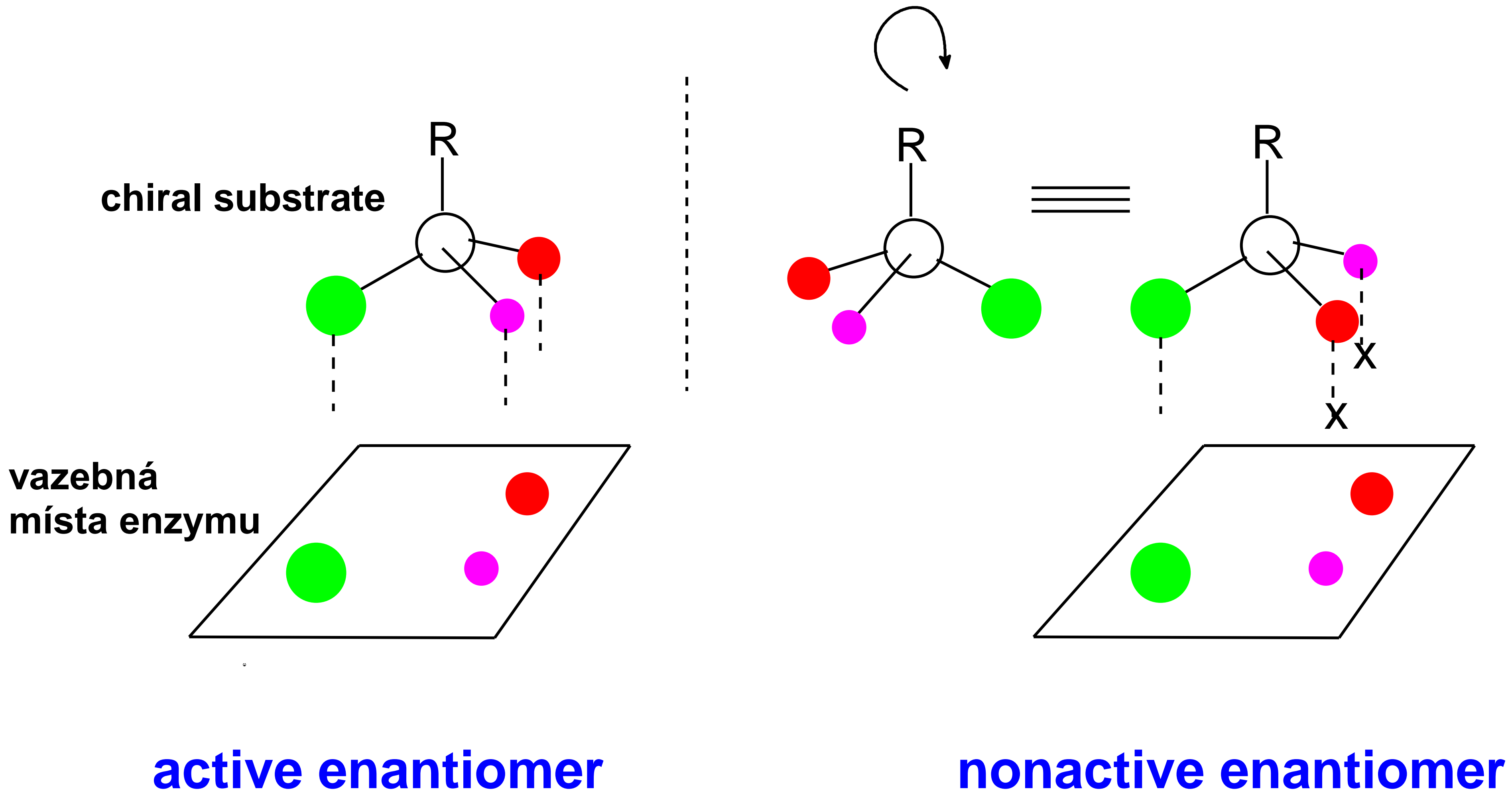
Binding of a chiral substrate (drug) into the active site of the enzyme



active enantiomer **nonactive enantiomer**

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The nomenclature of enzymes

trivial

extension **-in, ase**

pepsin, trypsin

amylase, lipase

recommended trivial names

system

terminal -**ase**

name contains information on:

1. substrate
2. type of response

examples of names

- Recommended trivial name: **alcohol dehydrogenase**

  System name: ethanol: NAD + -oxidoreductase

  Reaction: ethanol + NAD + $\rightarrow$ acetaldehyde + NADH + H +

- Recommended trivial name: **alanine aminotransferase (ALT)**

  System name: L-alanine-2-oxoglutarate aminotransferase

  Reaction: L-alanine + 2-oxoglutarate $\rightarrow$ pyruvate + L-glutamate

Classification of enzymes:

Six classes according to the type of reaction

1. **oxidoreductase**
2. **transferase**
3. **hydrolases**
4. **lyase**
5. **Isomerase**
6. **ligase**

TEST, examples

1. Oxidoreductases

- redox conversion of substrates
  various mechanisms, subclasses:
- **dehydrogenases,** transmit two H atoms (dehydrogenase)
- **oxidase**, transmitted electrons from substrate to substrate (cytochrome c oxidase)
- **oxygenase**, incorporated into the substrate O atom (monooxygenases, dioxygenase)
- **Peroxidase** (degradation of H_2O_2)

2. Transferases

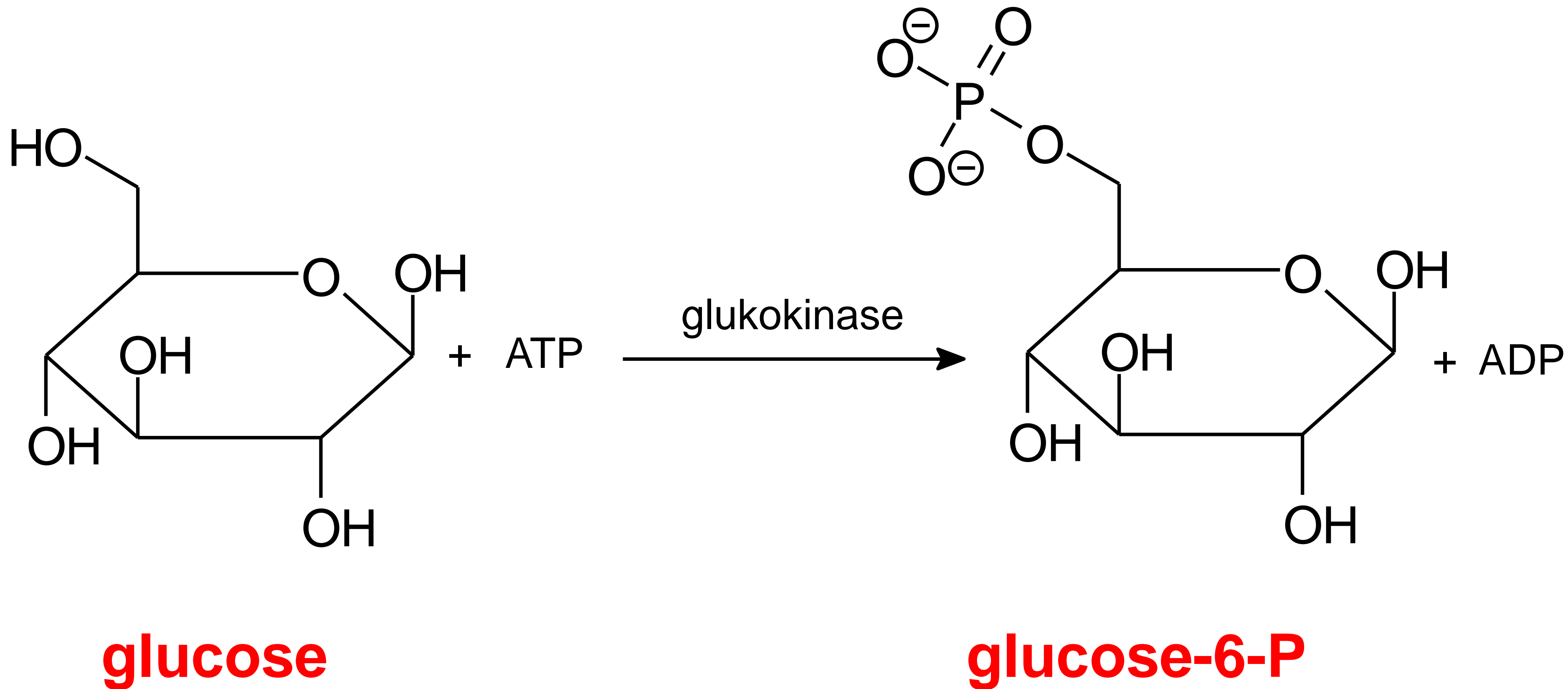
- transfer groups from one substrate to another

  aminotransferase, methyl, amino, glucose ...

  kinase - phosphorylation substrates $-PO_3^{2-}$ transfer

  from ATP to the OH group of the substrate

Example: Phosphorylation of Glu



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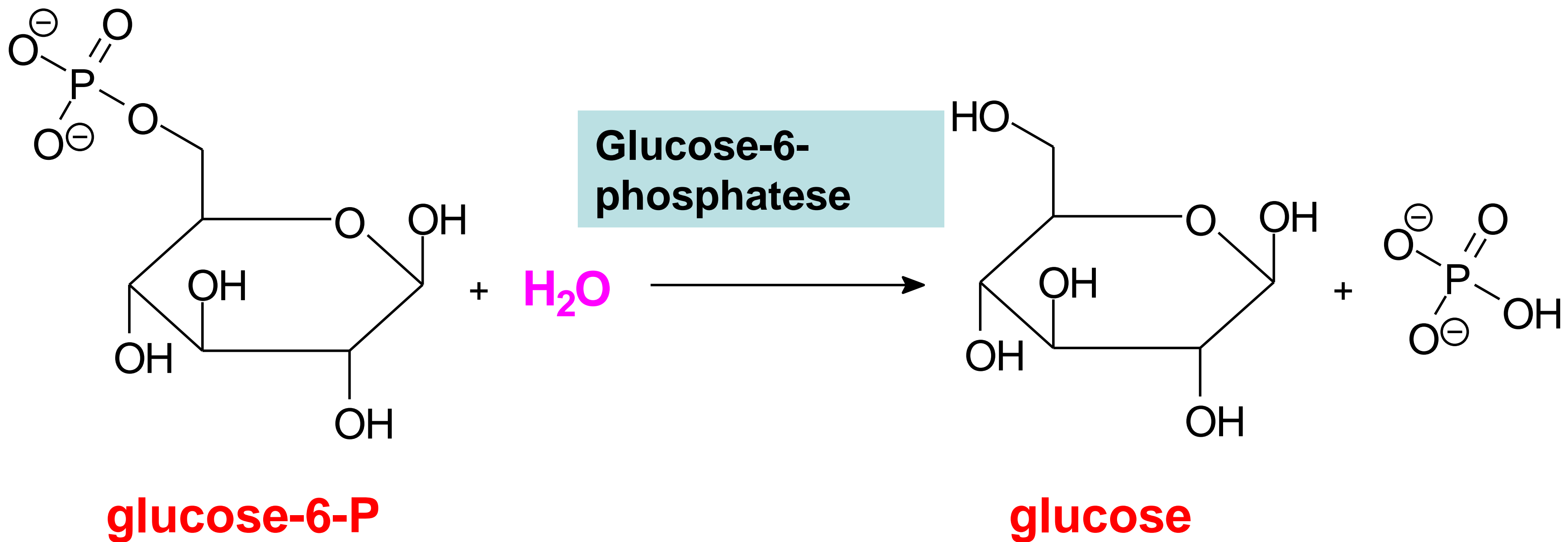
3. Hydrolases

- hydrolytically cleaves the links established by condensation
- peptide, ester, glycosidic
  protease, amylase, lipase, lysozyme
- **phosphatase** - cleaves phosphate esters

Example: Phosphatase



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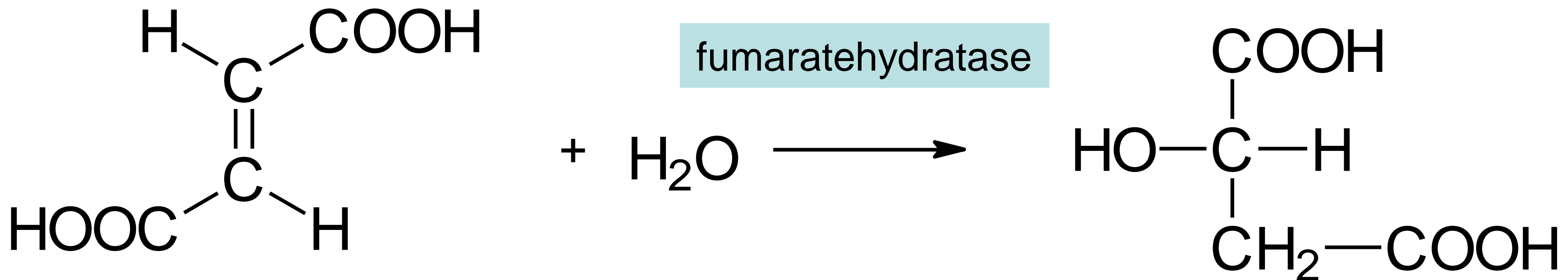
4. Lyases

- **non-hydrolytic cleavage** and **formation** of C-C bonds, C-O, C-N

- cleaved from the substrate or to bring it a small molecule (CO_2, H_2O)

- for example. **fumaratehydratase**

Example: Hydratation of fumarate on malate (fumaratehydratase)



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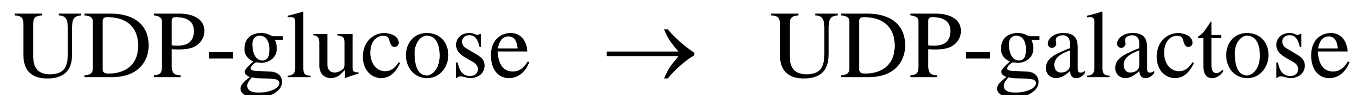
5. Isomerases

- **intramolecular rearrangements of atoms**

glucose-4-epimerase (epimerization of glucose)


UDP-glucose → UDP-galactose

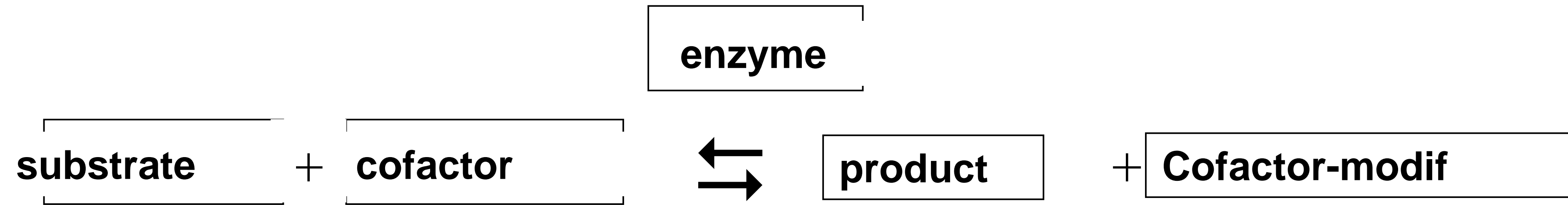
6. Ligase

- formation of energy-intensive ties with decomposition energy compounds (ATP)
-

pyruvate carboxylase

$$\text{pyruvate} + CO_2 + ATP + H_2O \rightarrow \text{oxalacetate} + ADP + P_i$$

Three different components in the enzyme reaction



1. Substrate (s) - a low molecular weight
2. cofactor - low molecular

} Directly interact

3. enzyme - high molecular weight, coordinates and accelerates the reaction

Note .: some reactions proceed without cofactor (eg. hydrolysis), the substrates can be a high molecular weight

Cofactors of enzymes

- low molecular weight non-protein compounds

  - transmit ***e-*** or ***2H*** --- ***oxidoreductase***

  - transfer of groups --- ***transferase***

  tightly bound - **prosthetic group**

  loosely coupled - **coenzymes (co-substrate)**

TEST

Coenzymes and prosthetic groups

Nomenclature

- ***Cofactor***: nonprotein component of enzymes
- Cofactor - a co-catalyst required for enzyme activity

1) Coenzyme - a dissociable cofactor, usually organic

2) Prosthetic group - non-dissociable cofactor

- Vitamin - a required micro-nutrient (organism cannot synthesize adequate quantities for normal health - may vary during life-cycle).
  - water soluble - not stored, generally no problem with overdose
  - lipid soluble - stored, often toxic with overdose.
- **Apoenzyme** - enzyme lacking cofactor (inactive)
- **Holoenzyme** - enzyme with cofactors (active)

TEST

Vitamins are precursors of cofactors

TABLE 8.9 Water-Soluble Vitamins

Vitamin	Coenzyme	Typical reaction type	Consequences of deficiency
Thiamine (B_1)	Thiamine pyrophosphate	Aldehyde transfer	Beriberi (weight loss, heart problems, neurological dysfunction)
Riboflavin (B_2)	Flavin adenine dinucleotide (FAD)	Oxidation–reduction	Cheliosis and angular stomatitus (lesions of the mouth), dermatitis
Pyridoxine (B_6)	Pyridoxal phosphate	Group transfer to or from amino acids	Depression, confusion, convulsions
Nicotinic acid (niacin)	Nicotinamide adenine dinucleotide (NAD^+)	Oxidation–reduction	Pellagra (dermatitis, depression, diarrhea)
Pantothenic acid	Coenzyme A	Acyl–group transfer	Hypertension
Biotin	Biotin–lysine complexes (biocytin)	ATP-dependent carboxylation and carboxyl-group transfer	Rash about the eyebrows, muscle pain, fatigue (rare)
Folic acid	Tetrahydrofolate	Transfer of one-carbon components; thymine synthesis	Anemia, neural-tube defects in development
B_{12}	5′-Deoxyadenosyl cobalamin	Transfer of methyl groups; intramolecular rearrangements	Anemia, pernicious anemia, methylmalonic acidosis
C (ascorbic acid)		Antioxidant	Scurvy (swollen and bleeding gums, subdermal hemorrhages)

Vitamins and cofactors of oxidodeductases

Vitamine	**Cofactors**	**Function of cofactors**
Niacin	NAD^+	acceptore 2H
Niacin	$NADPH + H^+$	donore 2H
Riboflavin	FAD, FMN	accceptore 2H
-----	tetrahydrobiopterin	donor 2H
-----	molybdopterin	e transport
-----	lipoate	acceptore 2H
-----	ubichinon	2 electron transport (and $2H^+$)
-----	hem cytochrom	1 electron transport
-----	nonhem Fe a S	1 electron transport
-----	**2** GSH	donore **2**H

Cofactors of oxidoreductases always exist in two forms

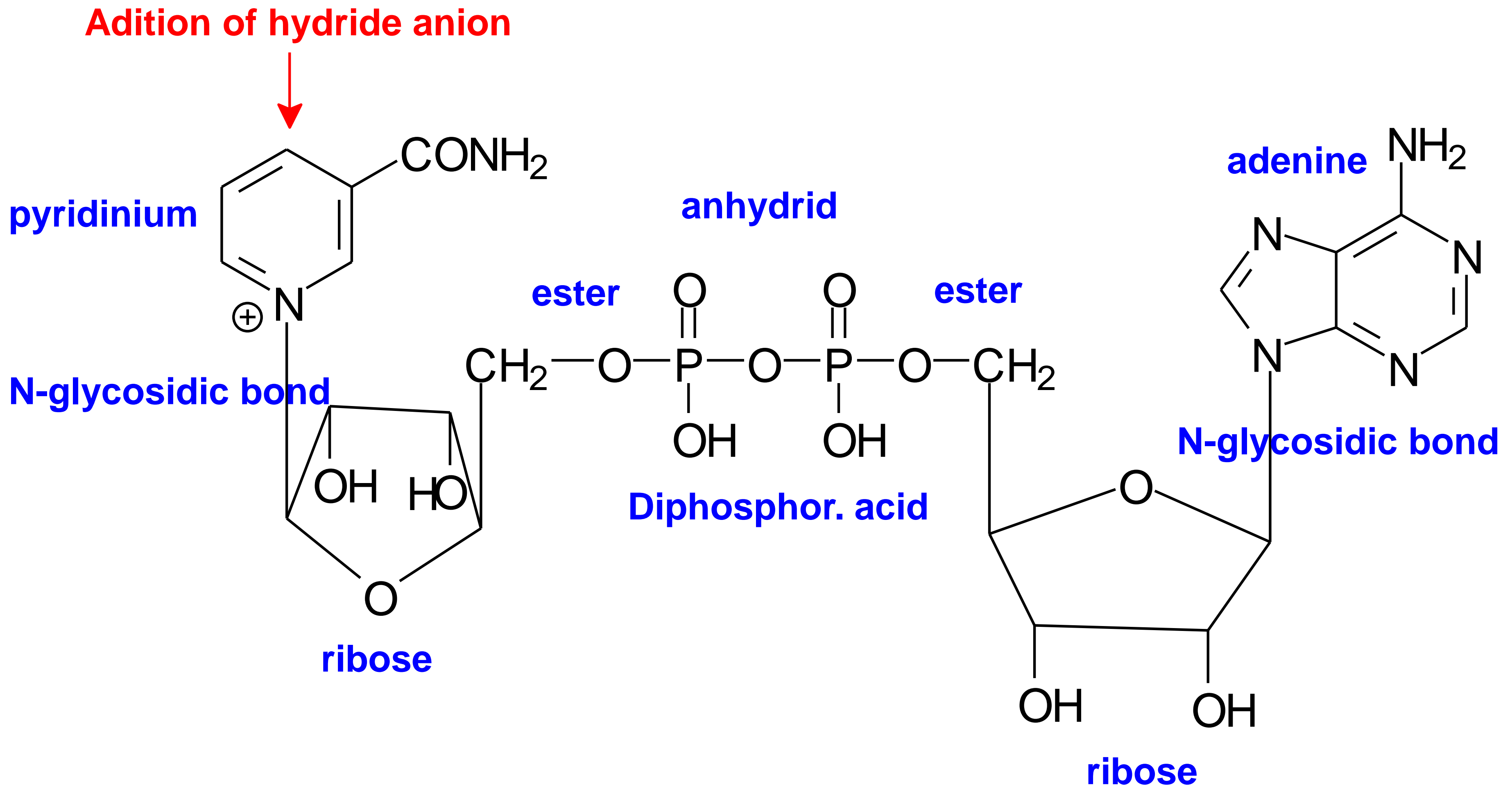
oxidized ⇆ reduced

form a redox couple

Nicotinic Acid/Nicotinamide Coenzymes

- These coenzymes are two-electron carriers
- They transfer hydride anion (H-) to and from substrates
- Two important coenzymes in this class:
- Nicotinamide adenine dinucleotide (NAD+)
- Nicotinamide adenine dinucleotide phosphate (NADP+)

Structure of NAD$^+$



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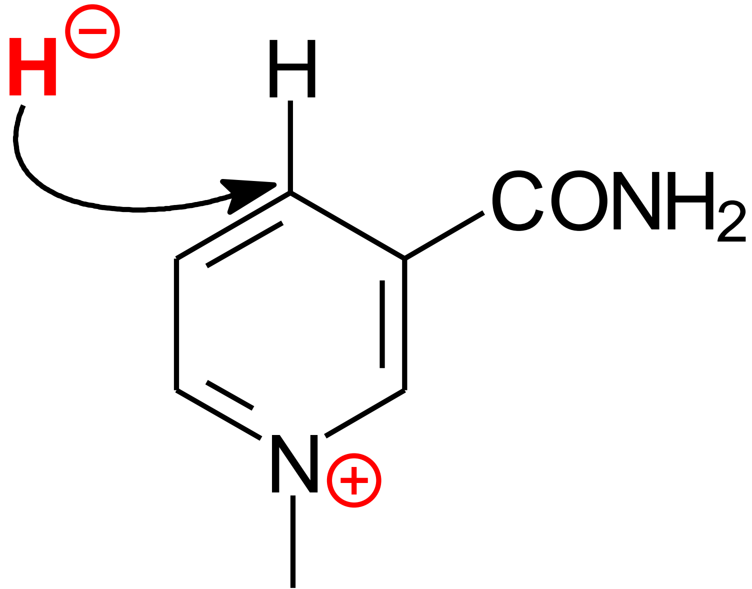
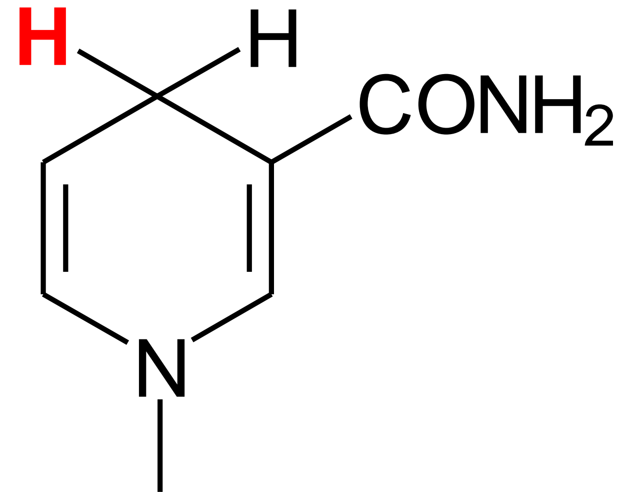
NAD^+ — NADH $(+H^+)$





Oxidized form — **Redused form**

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Dehydrogenation with NAD^+

- substrate loses two H atoms from the groups:

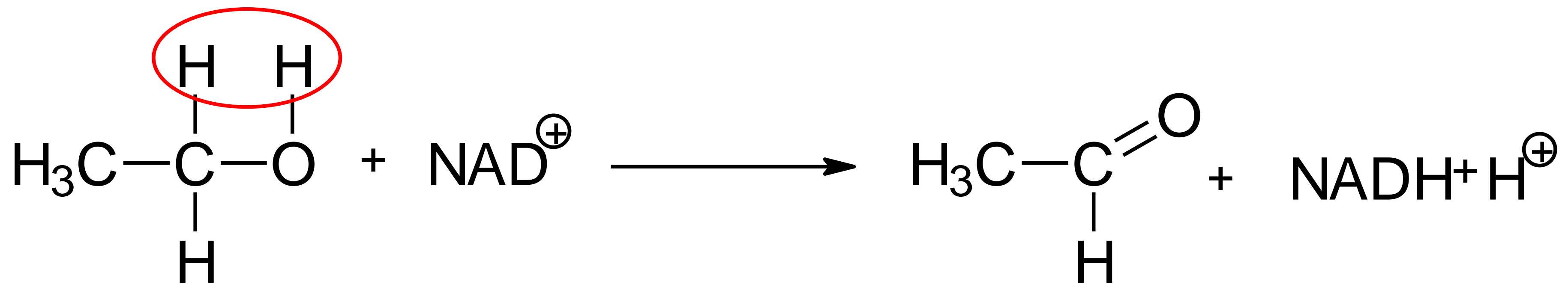
  primary alcohol group CH_2-OH

  secondary alcohol group> CH-OH

  a secondary amino group> CH-NH_2

  there is a double bond

Dehydrogenation of ethanol
(alcoholdehydrogenase)



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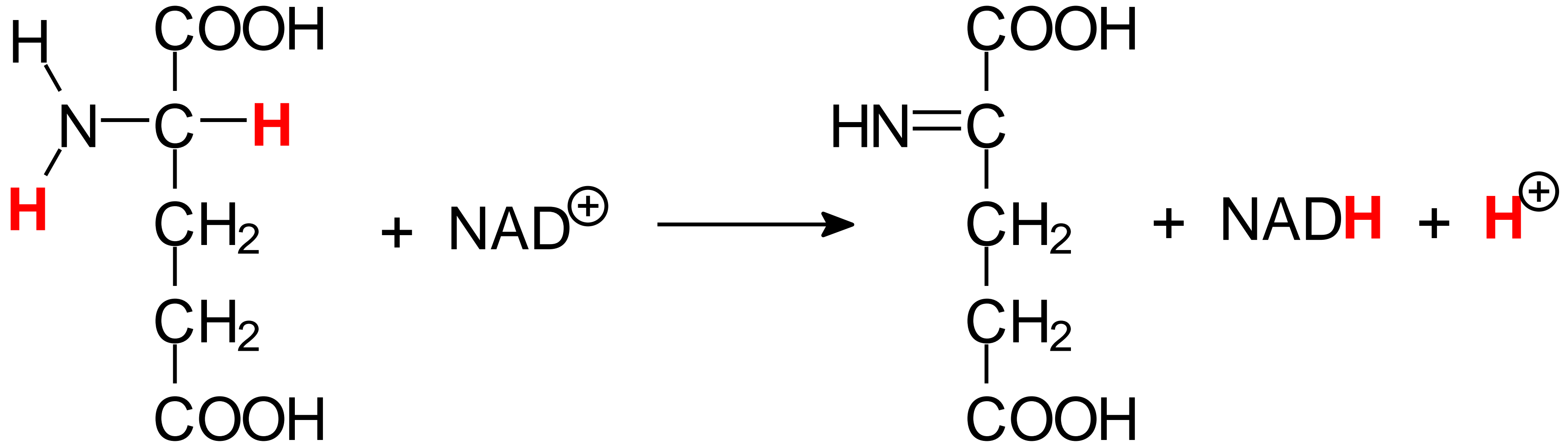
Dehydrogenation of glutamate
(glutamatedehydrogenase)



2-amino acid **2-imino acid**

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NAD^+-dependent enzyme

- **Citrate cycle**:
  isocitratedehydrogenase
  2-oxoglutaratedehydrogenase
  malatedehydrogenase

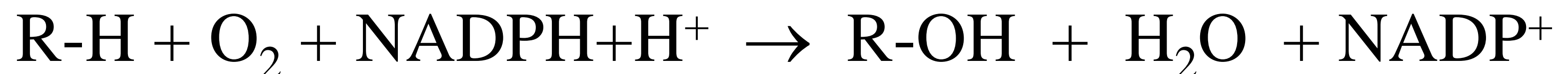
- **Glykolysis**:
  glyceraldehyd-3-P dehydrogenase
  laktatedehydrogenase

- **Detoxication of ethanol**:
  alcoholdehydrogenase
  acetaldehyddehydrogenase

NADPH + H^+ hydrogenation agent

- donor 2H hydrogenation
  reducing cofactor synthesis (FA, cholesterol)
  regeneration of GSH in erythrocytes!
  cofactor hydroxylation reactions:
  - cholesterol bile acids
  - kalciol -- calcitriol
  - xenobiotic -- hydroxylated xenobiotic

  general scheme hydroxylation:

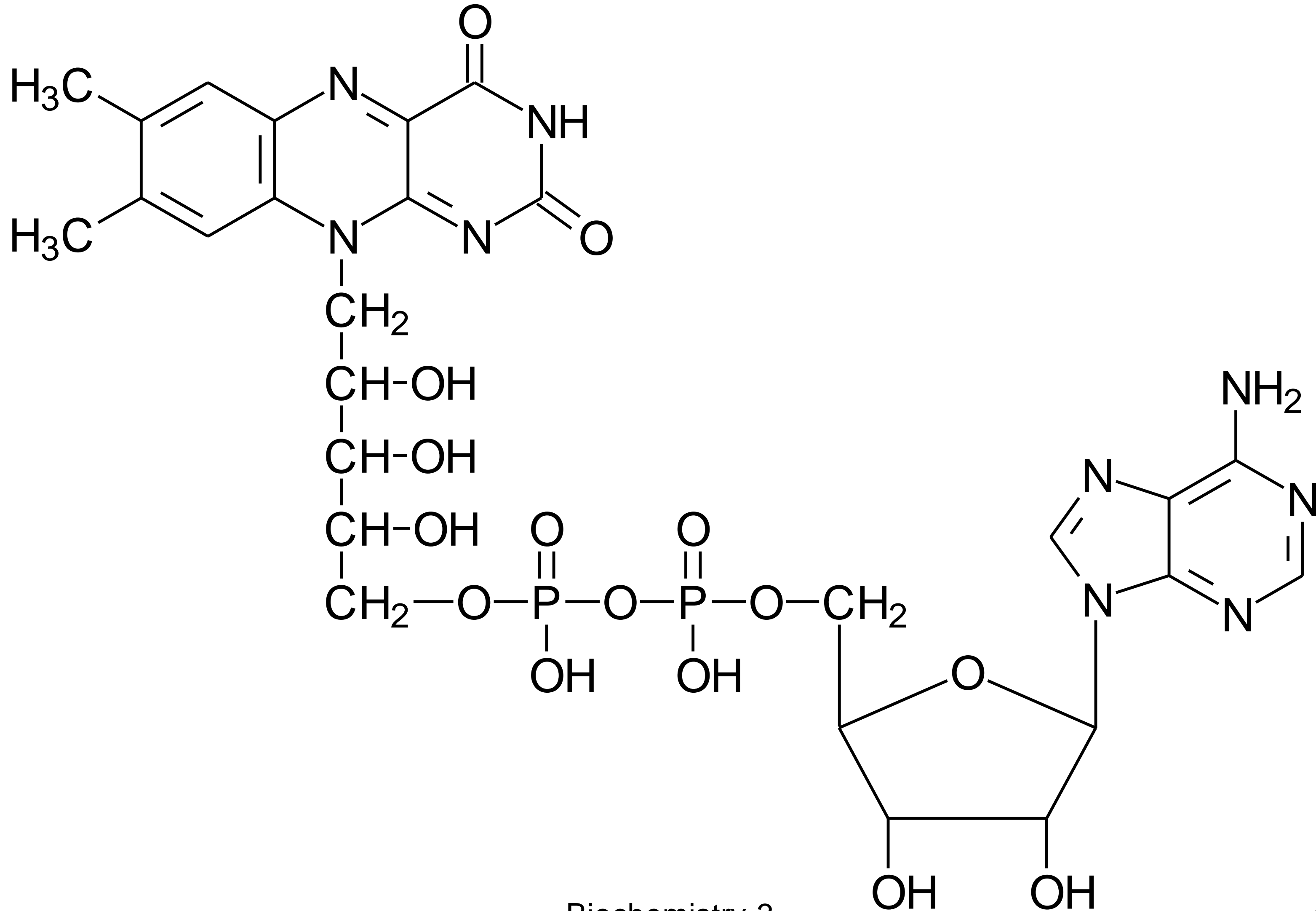


FAD is dehydrogenation agent

- <u>f</u>lavin<u>a</u>denin<u>d</u>inucleotide
- cofactor dehydrogenase
- Dehydrogenation of $-CH_2-CH_2-$ group
- 2H are bindint to 2N of riboflavine

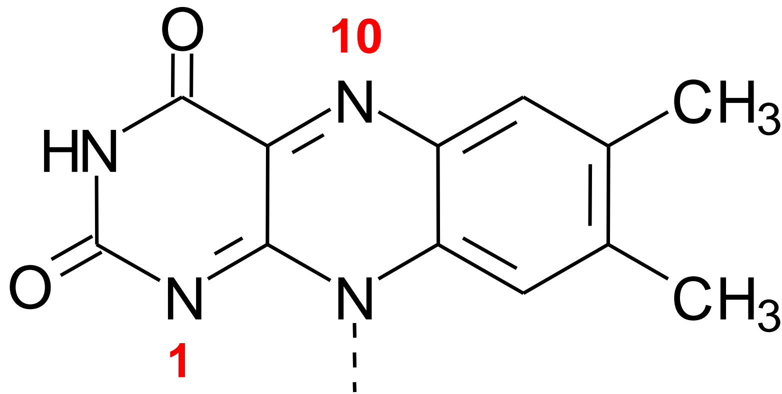
Prostetic group

Structure of FAD

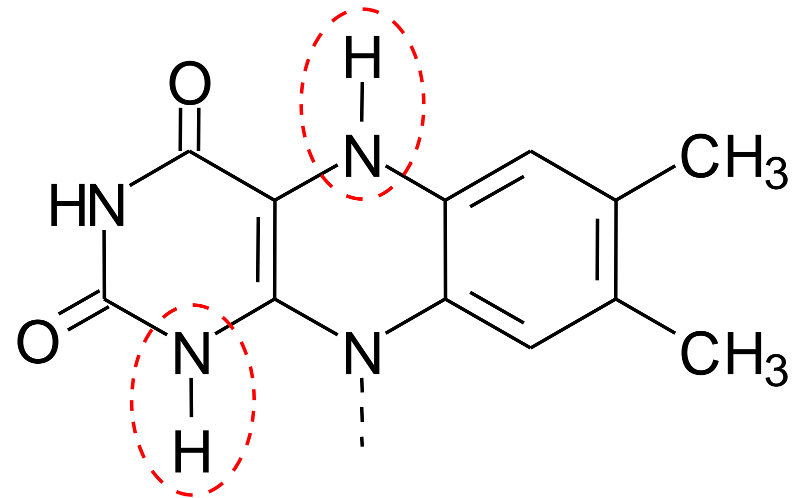


FAD

$FADH_2$



Oxidized form



Reduced form

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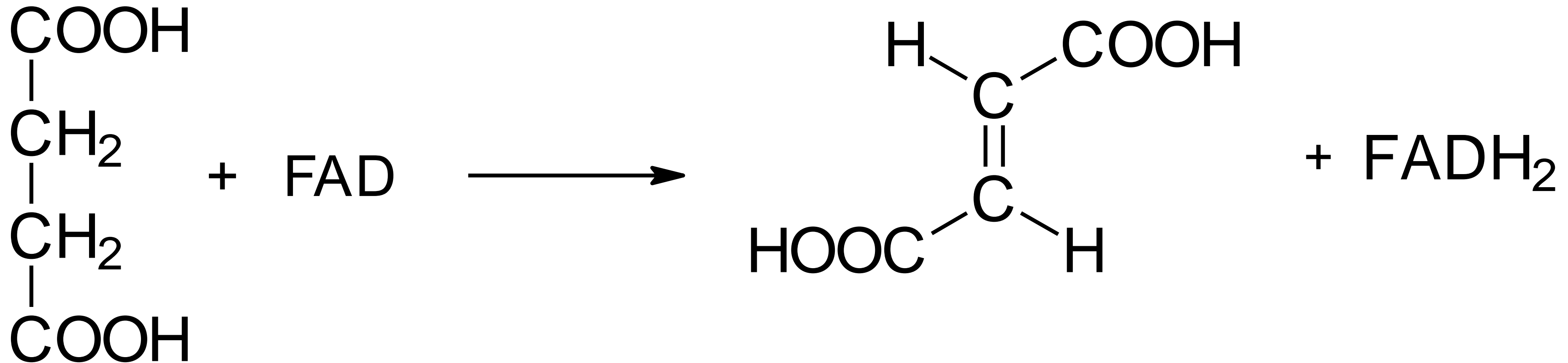
Dehydrogenation of succinate to fumarate



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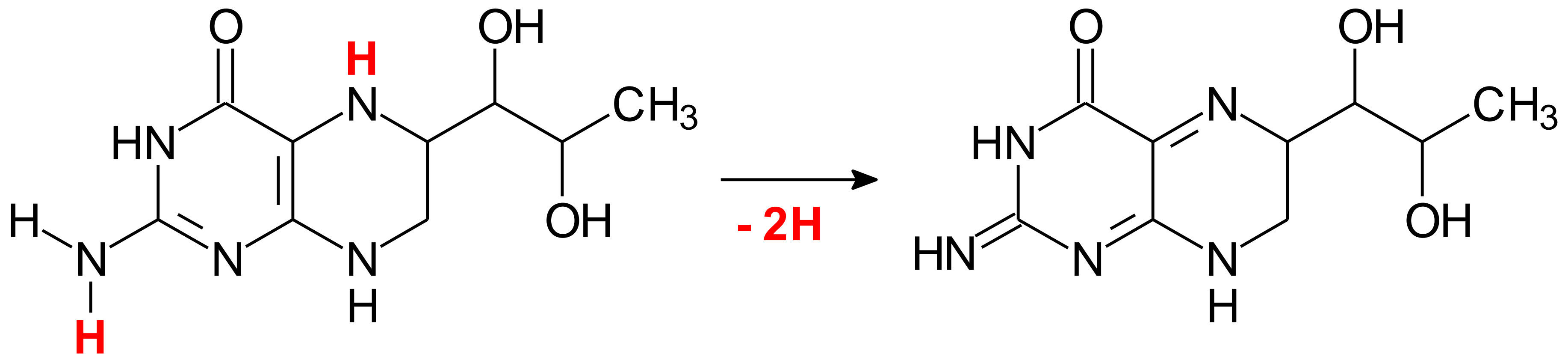
Tetrahydrobiopterine (BH_4) is hydrogenation agent

- coffactor of hydroxylation reactions
- gives 2H on O (water is produced)
- Oxidation on chinoid dihydrobiopterine

Dehydrogenace tetrahydrobiopterine



tetrahydrobiopterin (BH 4)

dihydrobiopterin (BH 2)

chinoid

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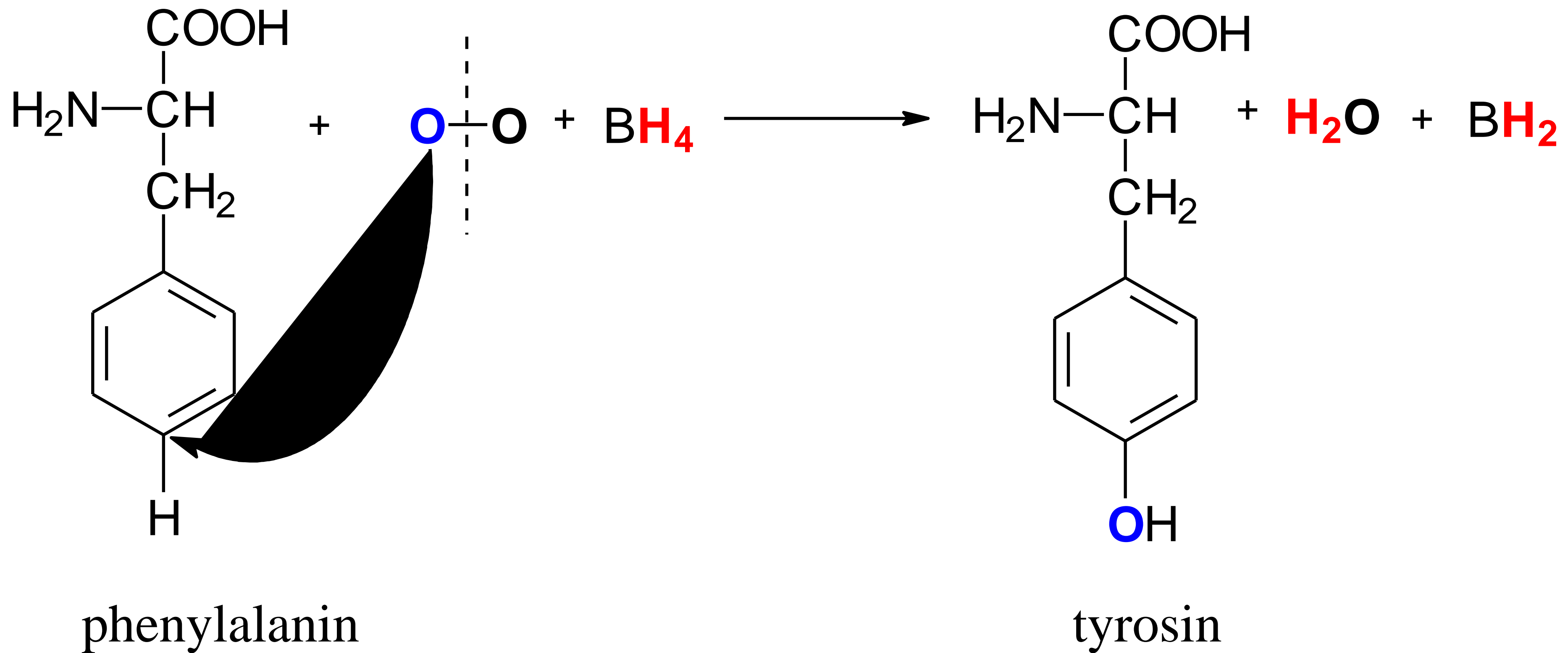
Hydroxylation of phenylalanine



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Coenzym Q (ubichinon)

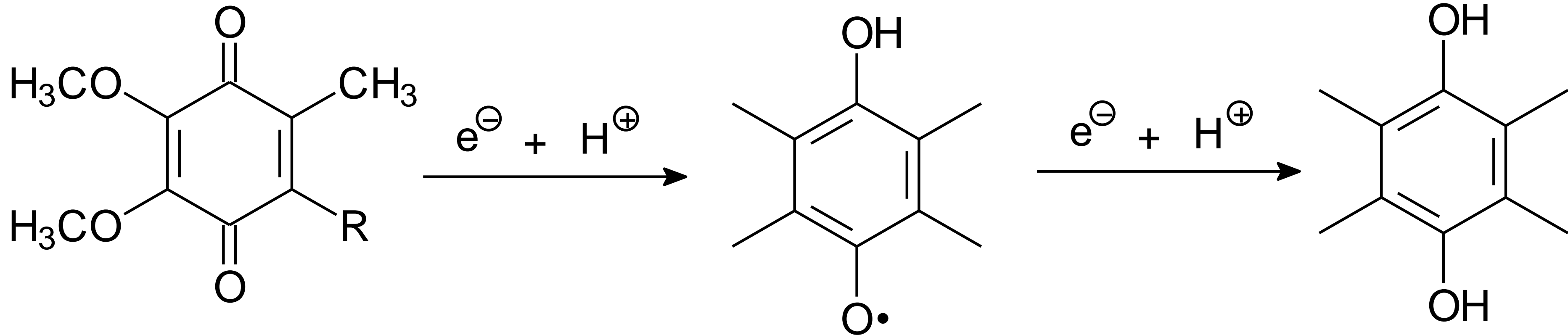
- a derivative of 1,4-benzoquinone

  - component of the respiratory chain

  - gradually accepts an electron and proton (2x)

  - reduces the semiubichinon and ubiquinol

Hydrogenation of ubichinone



ubichinon

no arom. ring

semiubichinon

arom. ring + radical

ubichinol

diphenol

elektrone (e^-) and protone (H^+) have different origine: elektrone from red. coffactors (=nutrients), H^+ from matrix of mitochond.

R = polyisoprenoid chain $\Rightarrow$ lipophilic

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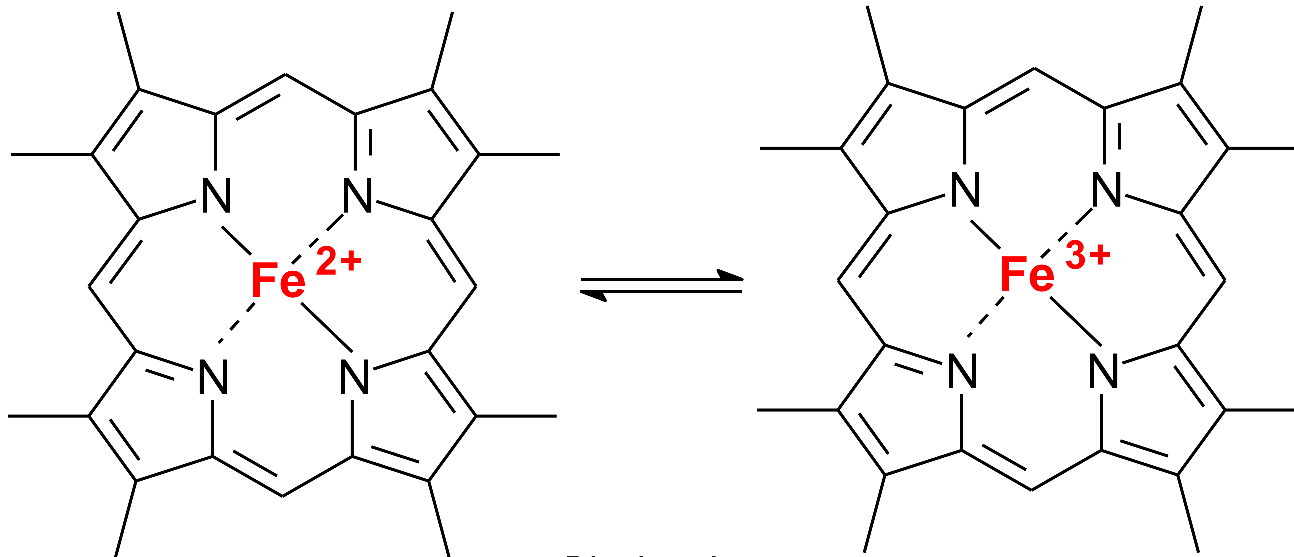
Hem of cytochromes

- transfers one electron
  cytochromes are hemoproteins,
  reversible transition occurs between Fe2 +
  and Fe3 +



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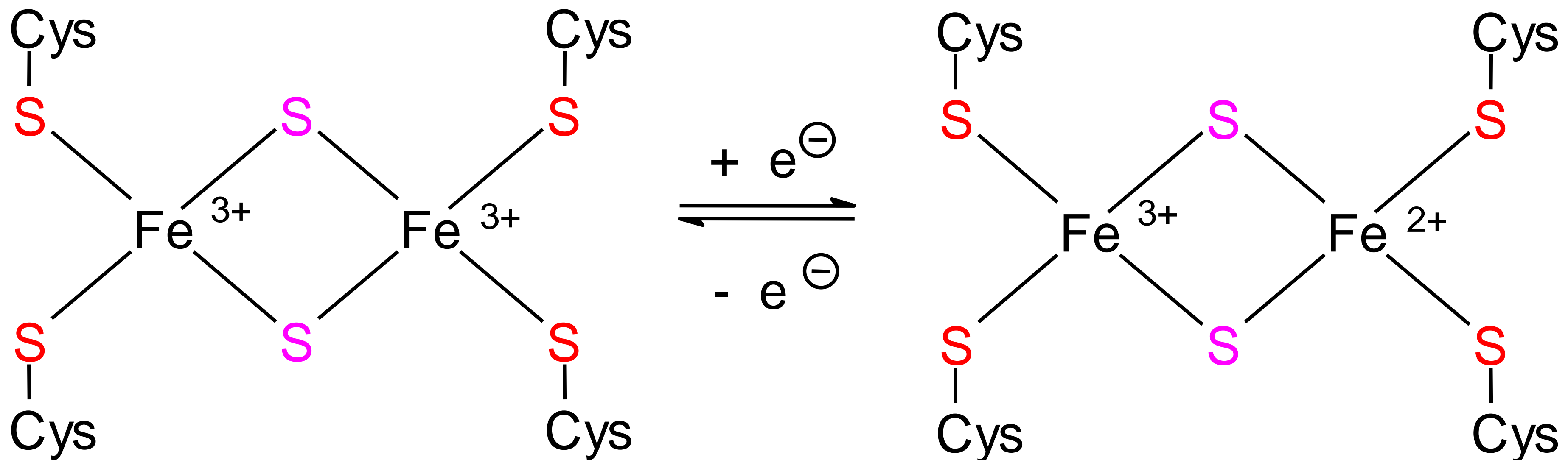
Non hem Fe – claster of Fe_2S_2



Oxidized form **Reduced form**

only one atome of Iron changes oxid. number

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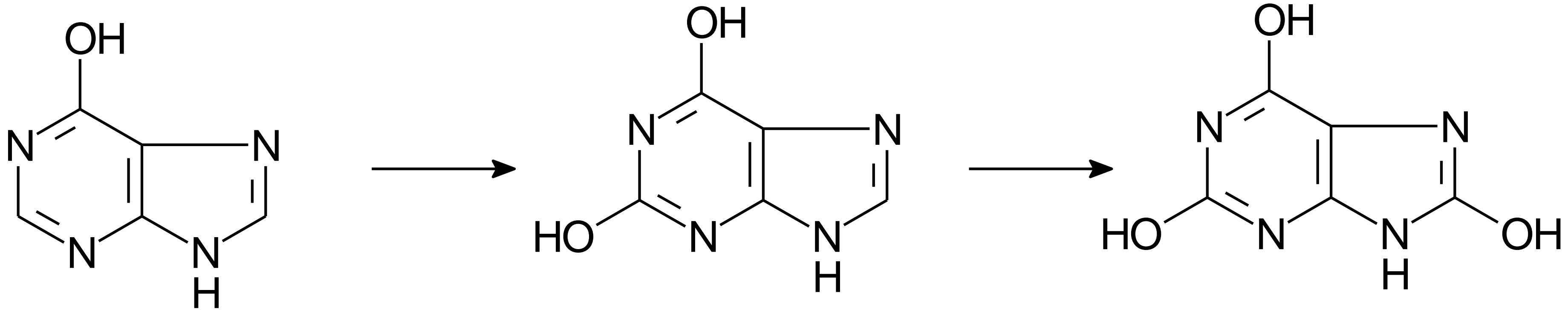
Molybdopterine

- the pteridine system heterocycle bonded with molybdenum oxygenase cofactor for example. xanthine oxidase, sulfitoxidase

Xanthinoxidase: oxygenation of purine



hypoxanthin xanthin uric acid.

Sulfitoxidase: formation of sulfate anion

cysteine

$$HSO_3^- + H_2O \rightarrow SO_4^{2-} + 3\,H^+ + 2\,e^-$$

plazma (0,5 mmol/l)
urine

ECT

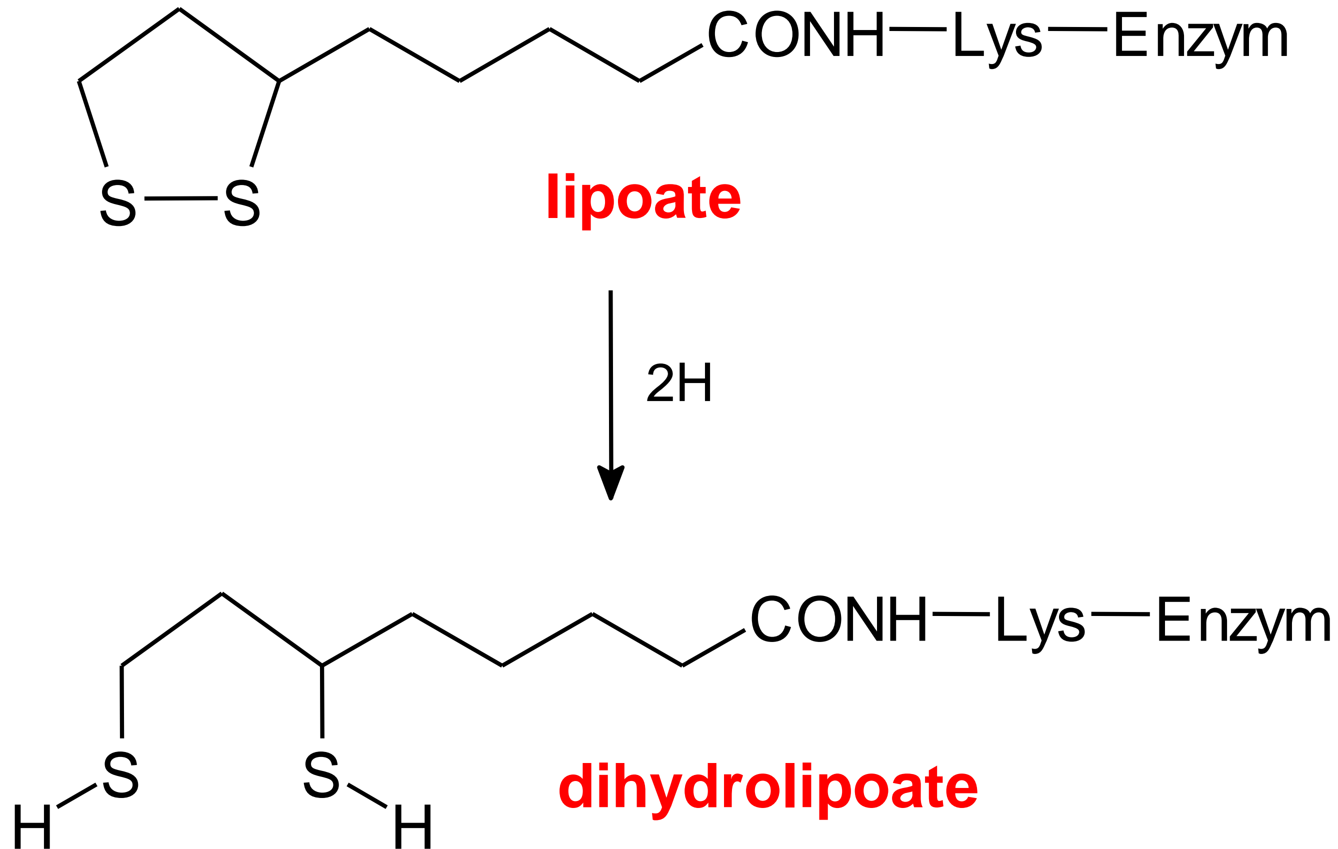
Reduction of Mo

Lipoate

- cyclic disulfide (S-S)

- 1,2-dithiolane-3-pentanoic acid
  - amide linkage to lysine enzyme
  - adoption 2H has two SH groups
  - part of a complex oxidative decarboxylation of 2-oxo acids (pyruvate, 2-OG)

Hydrogenation of lipoate

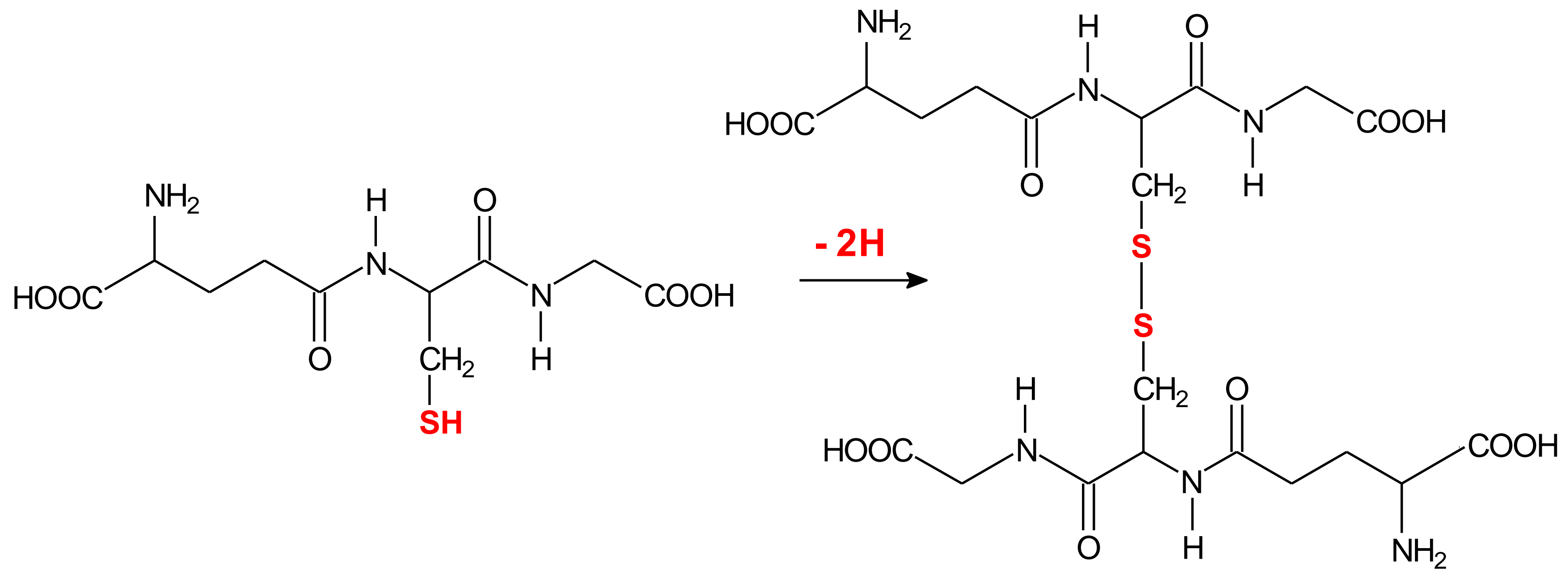


Glutathione (GSH)

- tripeptide
- γ-glutamylcysteinylglycin
- cofactor of glutathionperoxidase
- Reduction of H_2O_2 to water

⇨compounds with –SH reduction properties⇦

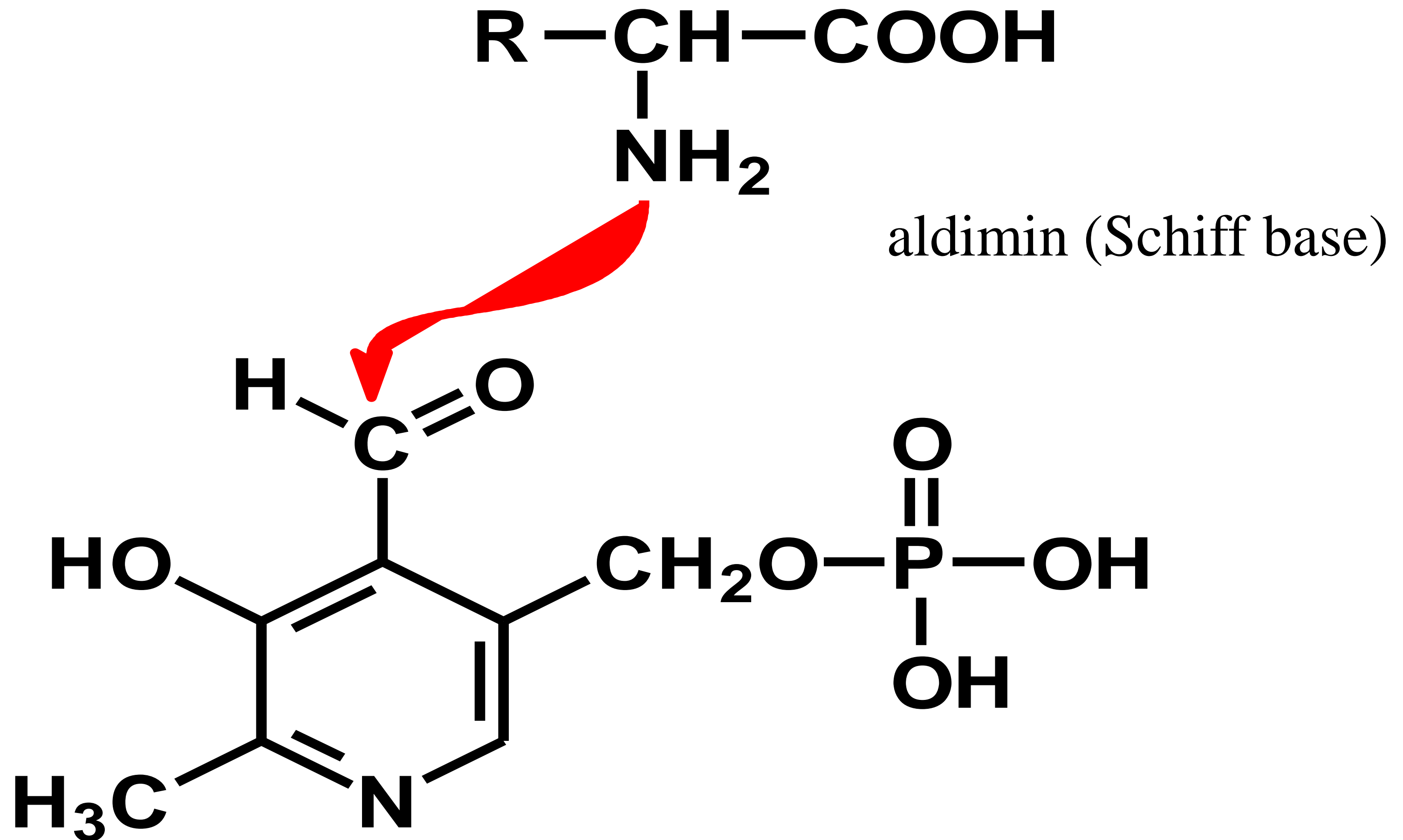
Dehydrogenation of 2 molecules of GSH



Vitamins and cofactors of transferases

Vitamine	cofaktor	group
---	ATP	$-PO_3^{2-}$
---	PAPS	$-SO_3^{2-}$
folic acid	H_4-folate	C_1 group
Biotin	carboxybiotin	CO_2
Thiamin	thiamindiP	aldehyd
Pyridoxin	pyridoxalP	$-NH_2$
Pantothen.acid	CoA-SH	acyl
---	dihydrolipoate	acyl
[Methionin]	SAM	$-CH_3$
cyanokobalamin	Methylcobalamin	$-CH_3$

Pyridoxalphosphate is cofactor of transaminases



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Scheme of 2 phases of transamination

AA

R–CH–COOH
NH_2

pyridoxal

$- H_2O$

R–CH–COOH
N
CH

Schiff base

R–C–COOH
N
CH_2

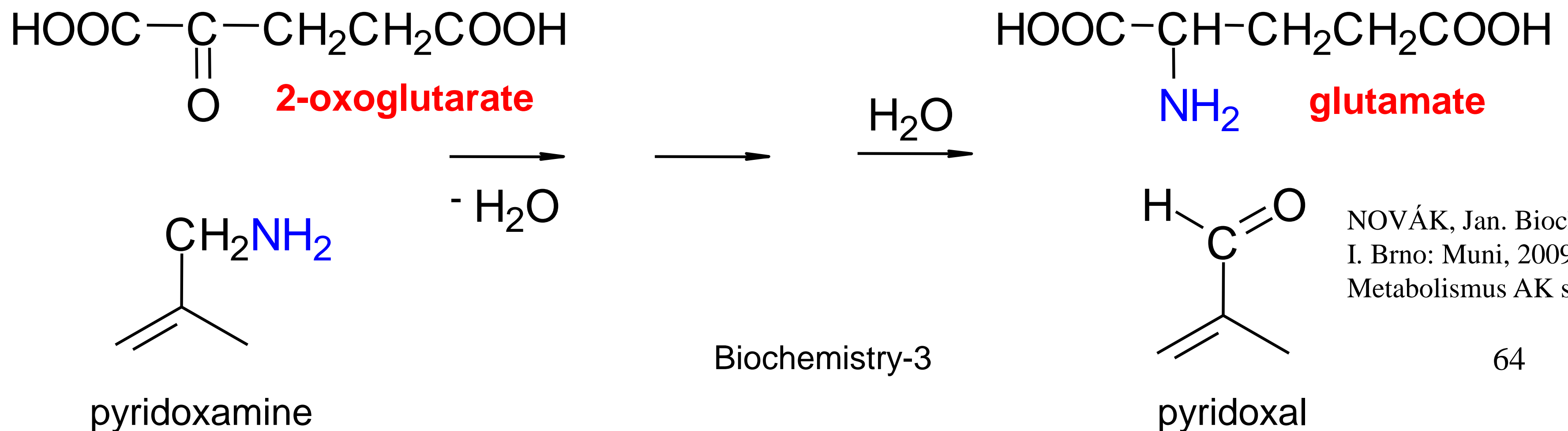
H_2O

R–C–COOH
O

2-Oxo-acid

CH_2NH_2

pyridoxamin



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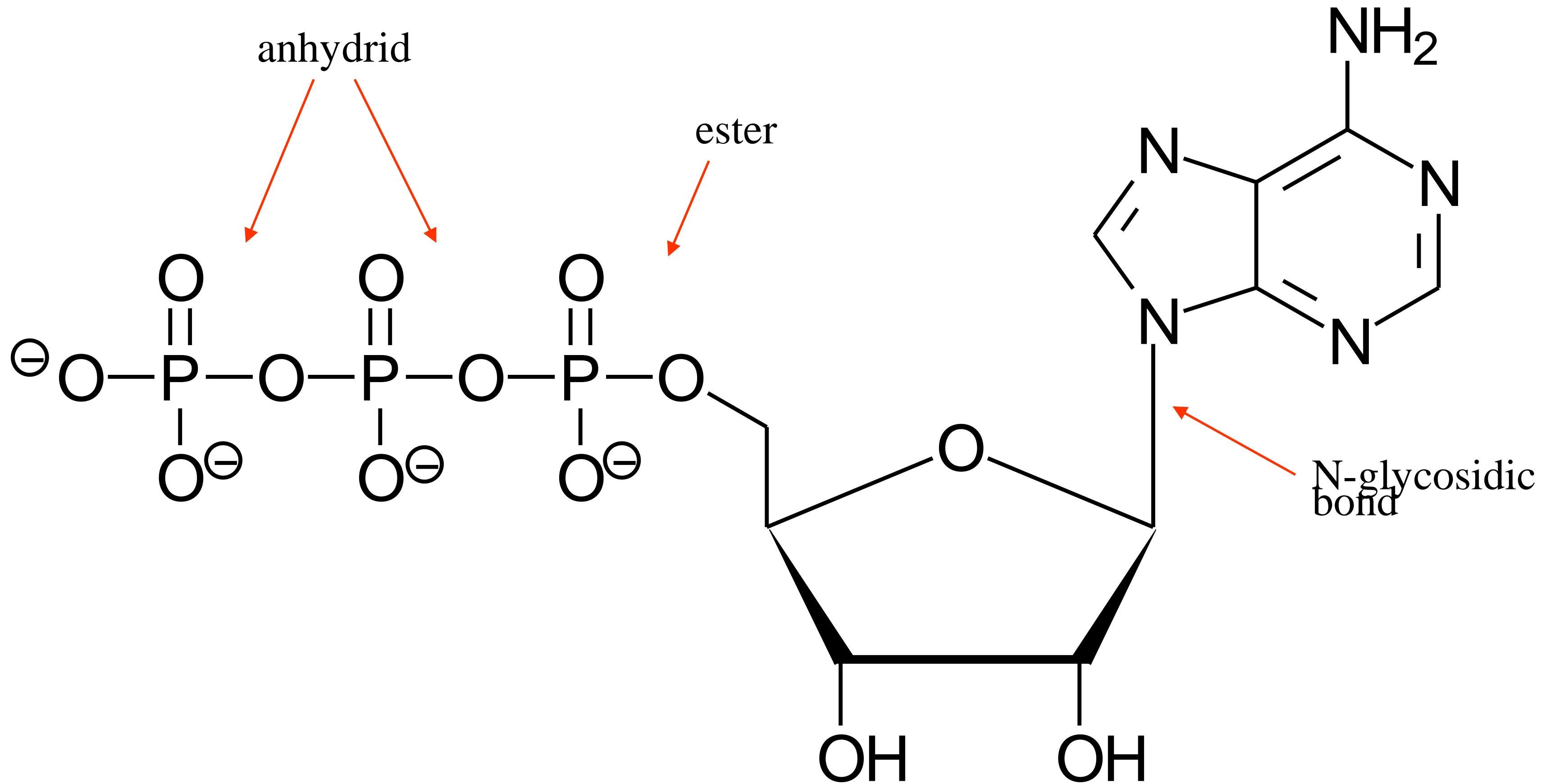
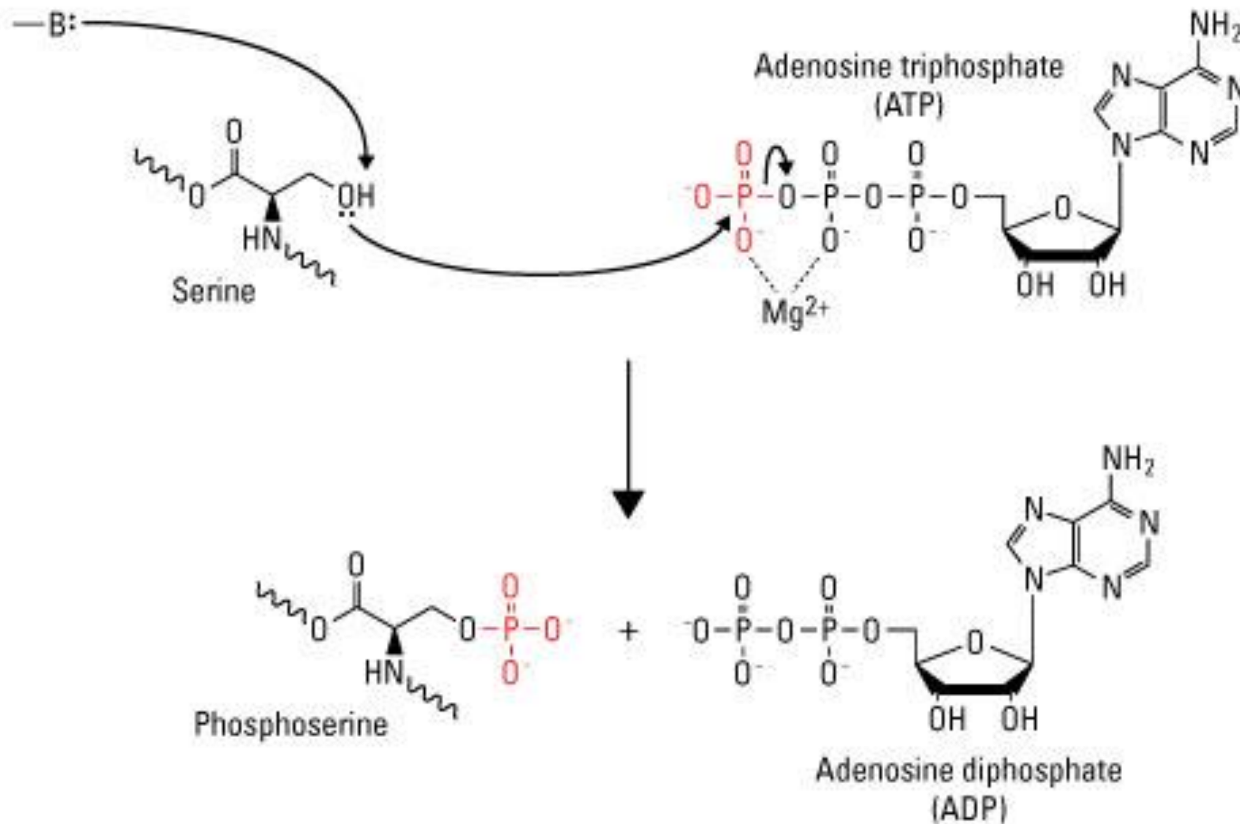
ATP (adenosintriphosphate)

- 2 importances :
- macroergic compound
- cofactor of kinases - phosphorylation reagent

Structure of ATP



Phosphorylation of substrate



http://www.piercenet.com/media/Serine%20Phosphorylation.jpg

PAPS is the sulfation agent

- 3'-5'-fosfoadenosin

  phosphosulfate

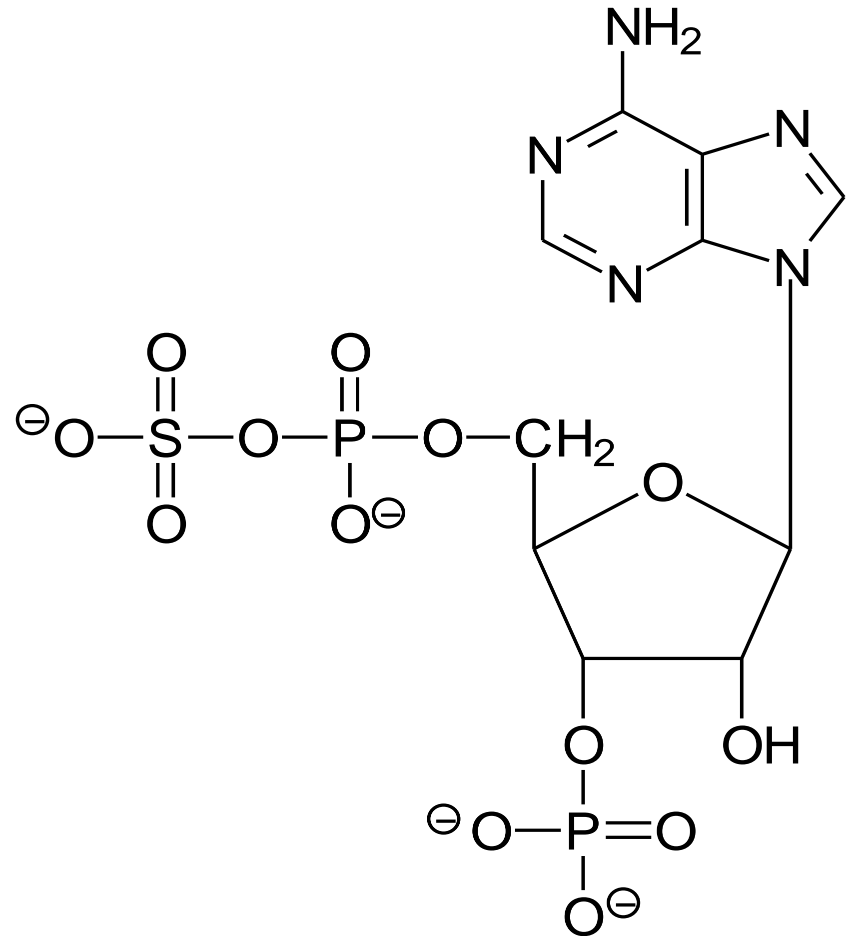
  - mixed anhydride of H2SO4

  and H3PO4

  - esterification of the

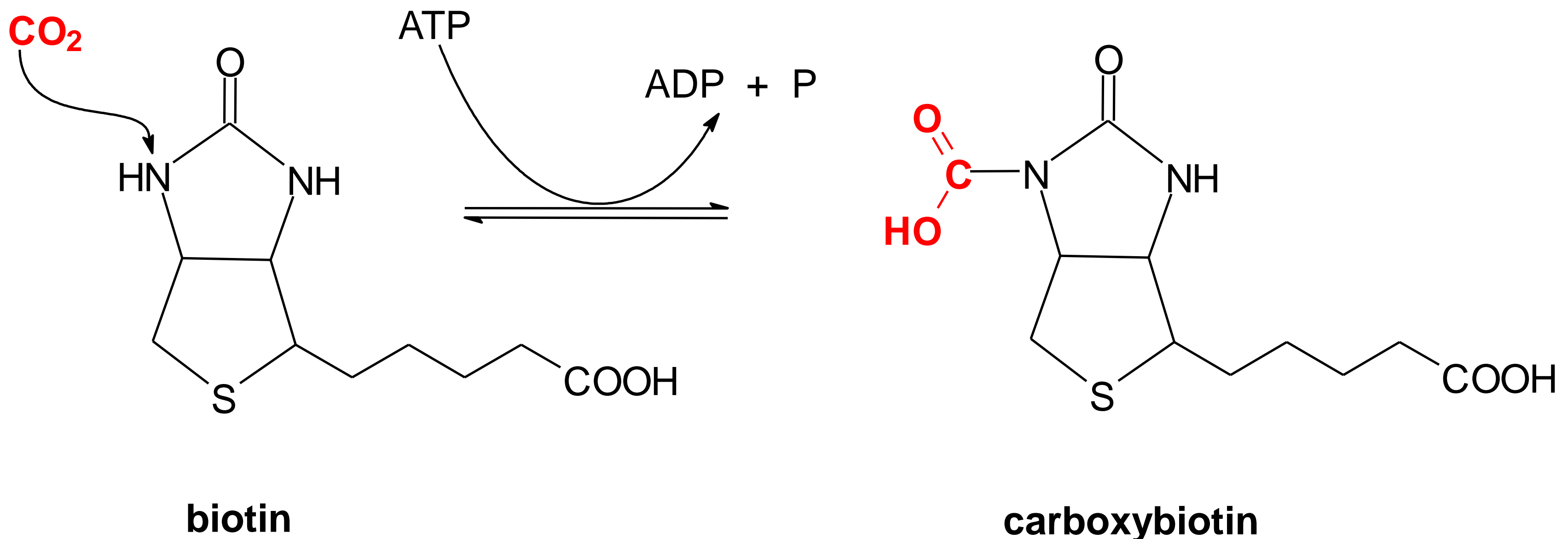
  hydroxyl groups of the acid.

  sulfuric



Carboxybiotin

- cofactor for carboxylation reactions carboxylation of biotin requires ATP



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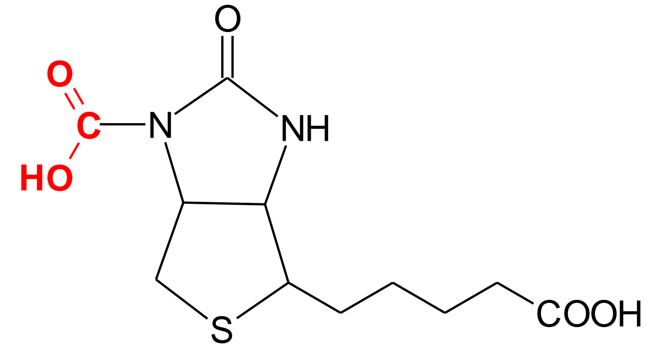
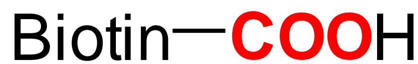
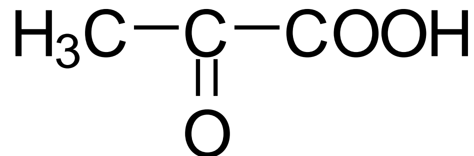
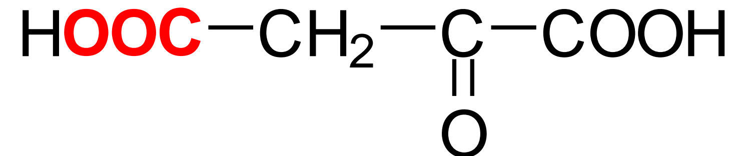
carboxybiotin is cofactor of carboxylation reactions





pyruvatcarboxylase





pyruvate

oxalacetate

Distinguish

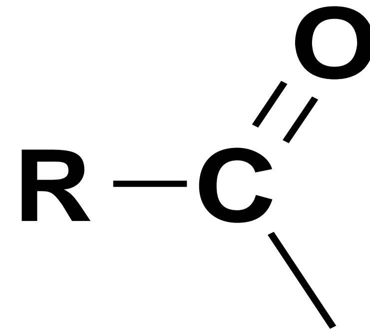
- **Carboxylation**
- carboxybiotin
- **Decarboxylatione**

enzymatic (*AA* - pyridoxalphosphate, 2-oxo acid. - TDP)

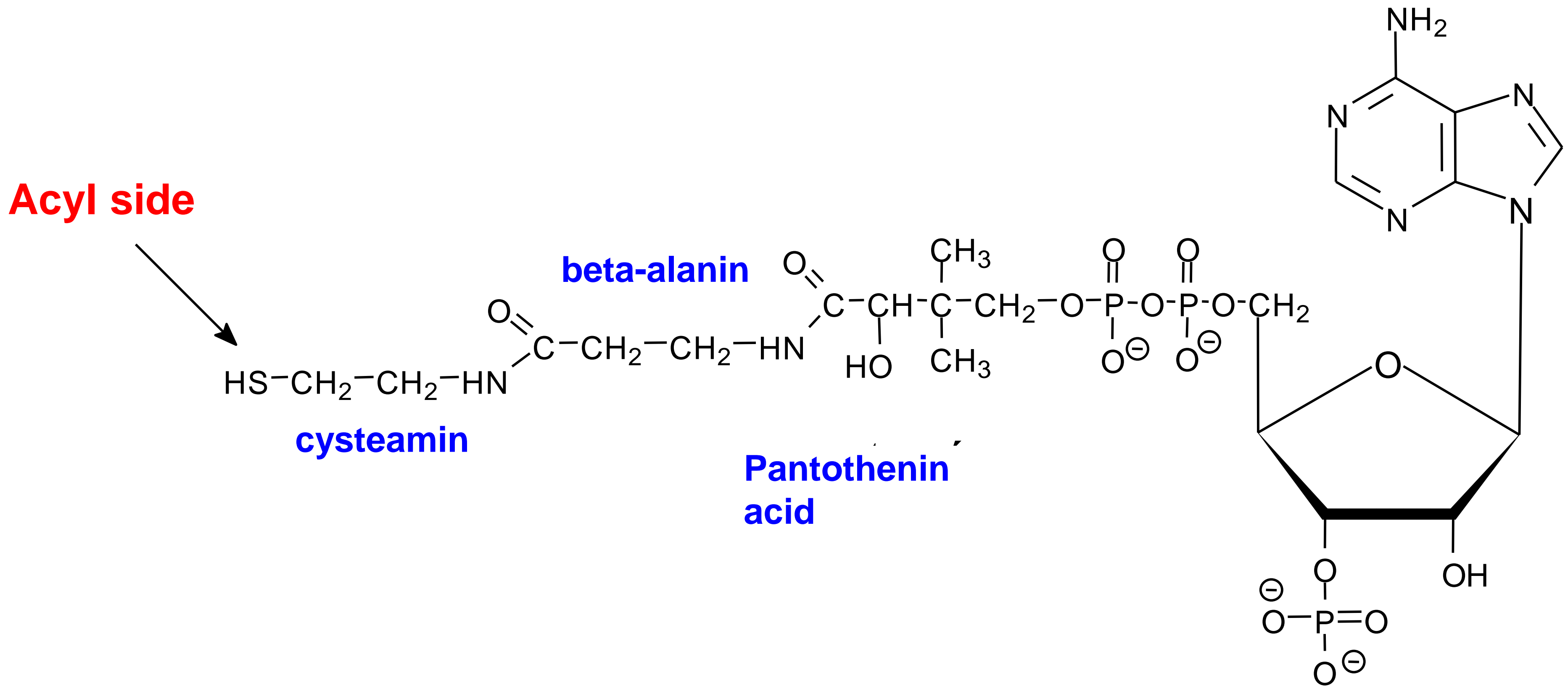
- non enzymatic (spontanous, without enzyme and cofactor, ex. acetoacetate → aceton)

Coenzym A (CoA-SH)

- transfers the acyl
  - bonded to the sulfur atom
  thioester bond
  - acyl-CoA is activated acyl
  - eg. acetyl-CoA



Coenzyme A



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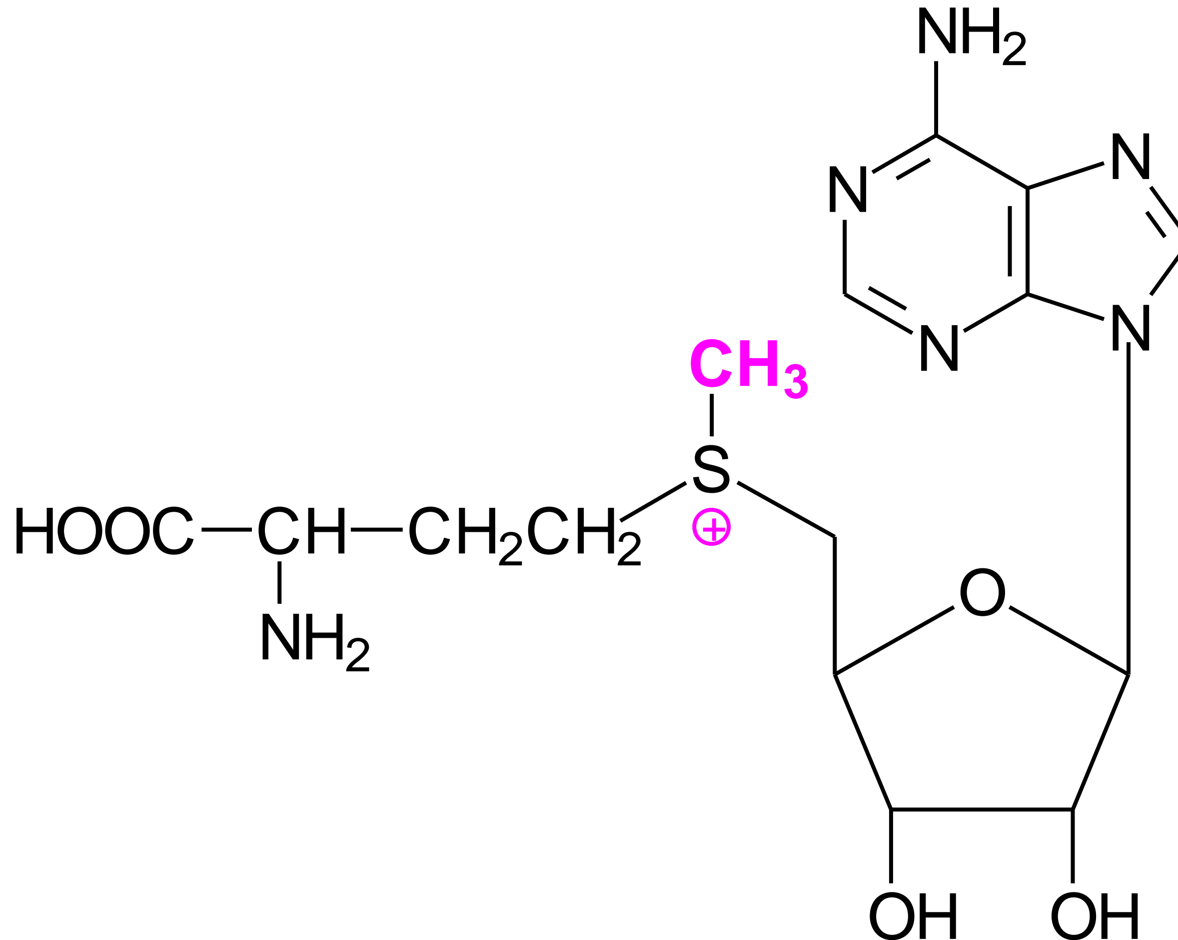
S-Adenosylmethionine (SAM)

- "Active methyl"
- S with 3 bonds
- cofactor methylation
- enzymes for example: phosphatidylcholine phosphatidylethanolamine →
  methionine from homocysteine arises

S-Adenosylmethionin (SAM)



Tetrahydrofolate

- Transport (C1) fragments
  - bind to the nitrogens N5 and / or N10
  - purine biosynthesis, methylation uracil
  - C1 fragments of the redox state:

- reduced: methyl **-CH_3**
- Mild reduced: methylen **-CH_2-**
- oxidized:

  formyl **-CHO** formimino **-CH=NH**

  methenyl **-CH=**

Sources C1 residues

- Trp catabolism: formate → formyl

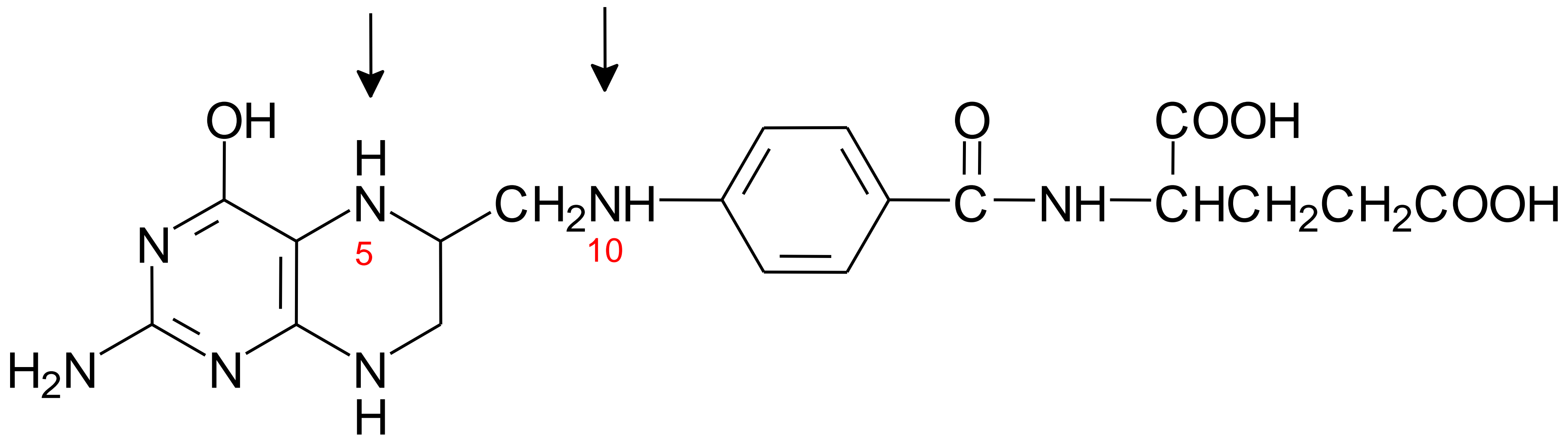
  His catabolism: formimino → methenyl

  Catabolism Ser: hydroxymethyl methylene →

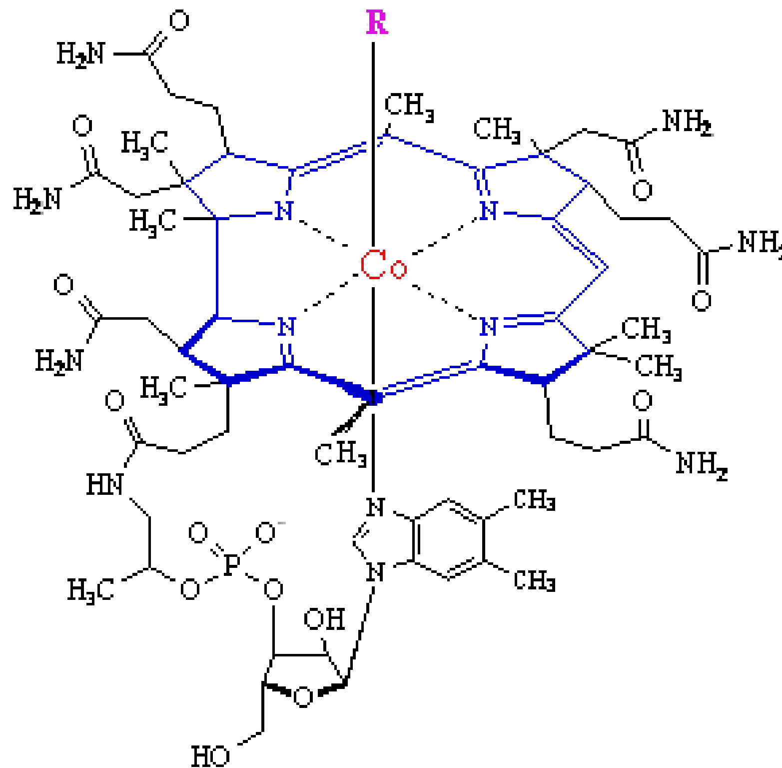
  Catabolism of Gly: methylene

  Methionine → SAM → methyl + homocysteine

Tetrahydrofolate

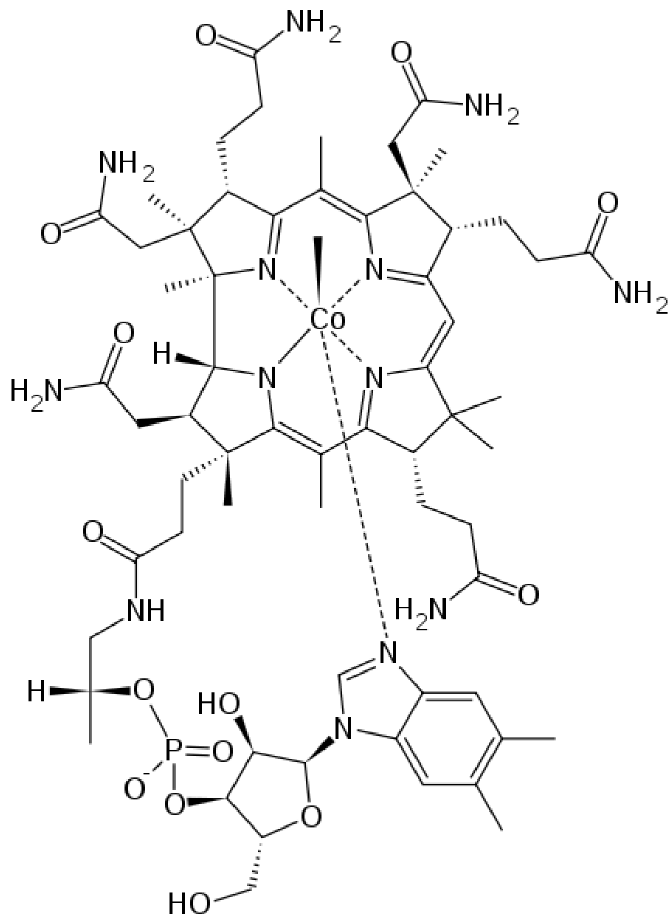


Vitamin B_{12} cyanocobalamine and/or hydroxocobalamine



http://www.chm.bris.ac.uk/motm/vitaminb12/b12.gif

Cofactor is methylcobalamin



- methylation reactions
- remethylation of homoCys to Met

- hydroxocobalamin - treatment of cyanide poisoning

  binds to toxic cyanide anions

  cyanocobalamin intravenous infusion

http://www.methylcobalamininfo.com/wp-content/uploads/2013/08/methylcobalamin2.png

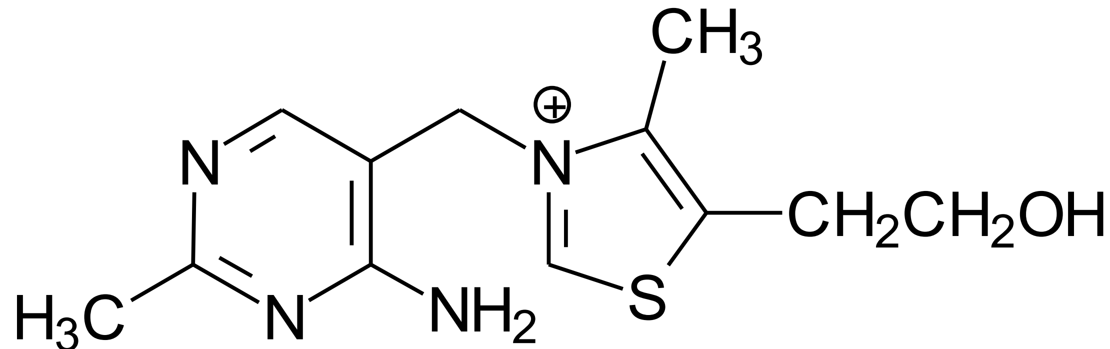
Two reactions with B_{12}

- S-methylation of homocysteine = regeneration, methyl is withdrawn from methyl tetrahydrofolate (and thereby creating H4 folate)

- propionyl-CoA $\rightarrow$ succinyl-CoA

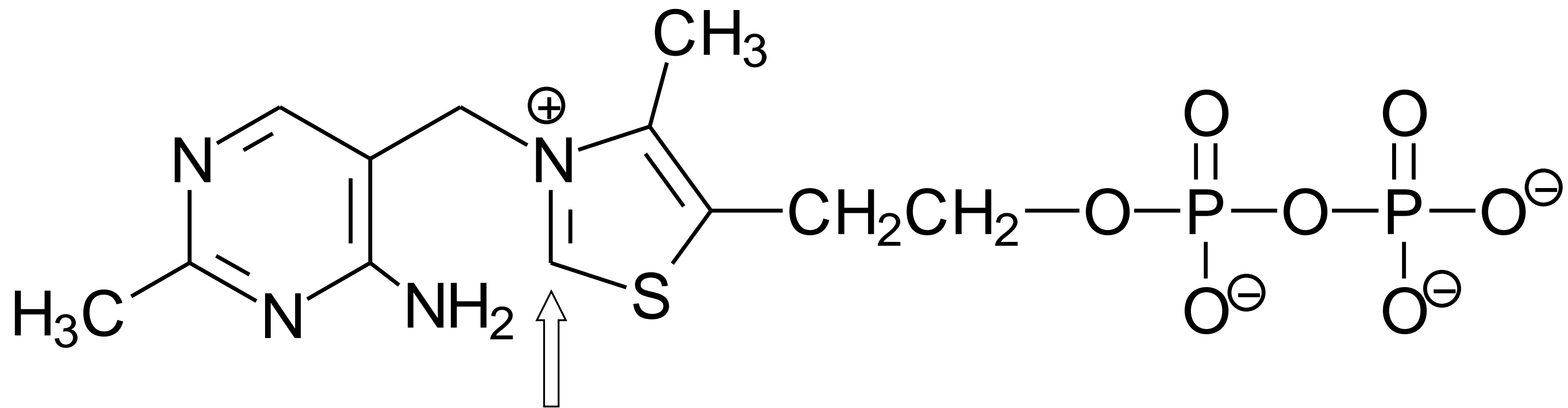
B_{12} necesary for regneration of tetrahydrofolate

Thiamin is vitamin B_1



- cofactor is thiamindiphosphate (TDP) oxidative decarboxylation of pyruvate,
- 2-oxoglutarate
  transfers so. activated aldehyde
  pyruvate → acetyl-CoA
- 2-OG → succinyl-CoA (CC)

Thiamindiphosphate (TDP) is cofactor of oxidative decarboxylation of pyruvate



Binding of pyruvate for decarboxylation

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The mechanism of enzyme reactions, metalloenzymes, kinetics, activity, enzymes in medicine

Active site of enzyme

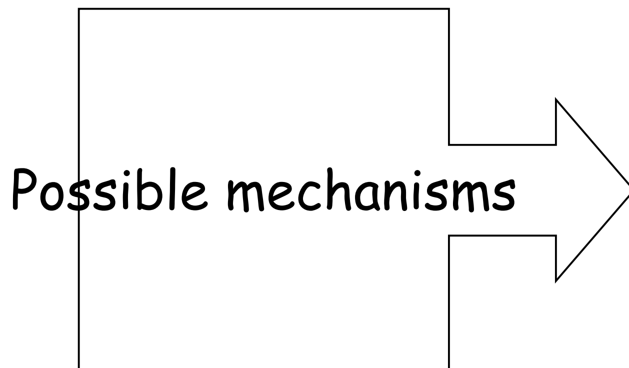
- small portion of the molecule,
- the three-dimensional arrangement
  deep slot (eg. amylase),
  surface depression
- Attending side chains AA distant in the primary structure
- Protein flexibility allows adaptation induced conformation corresponding substrate

Binding of substrate

- substrate binding to the active site causes conformational change of the molecule corresponding to the enzyme - induced adaptation
  creates a complex enzyme-substrate

catalytic groups

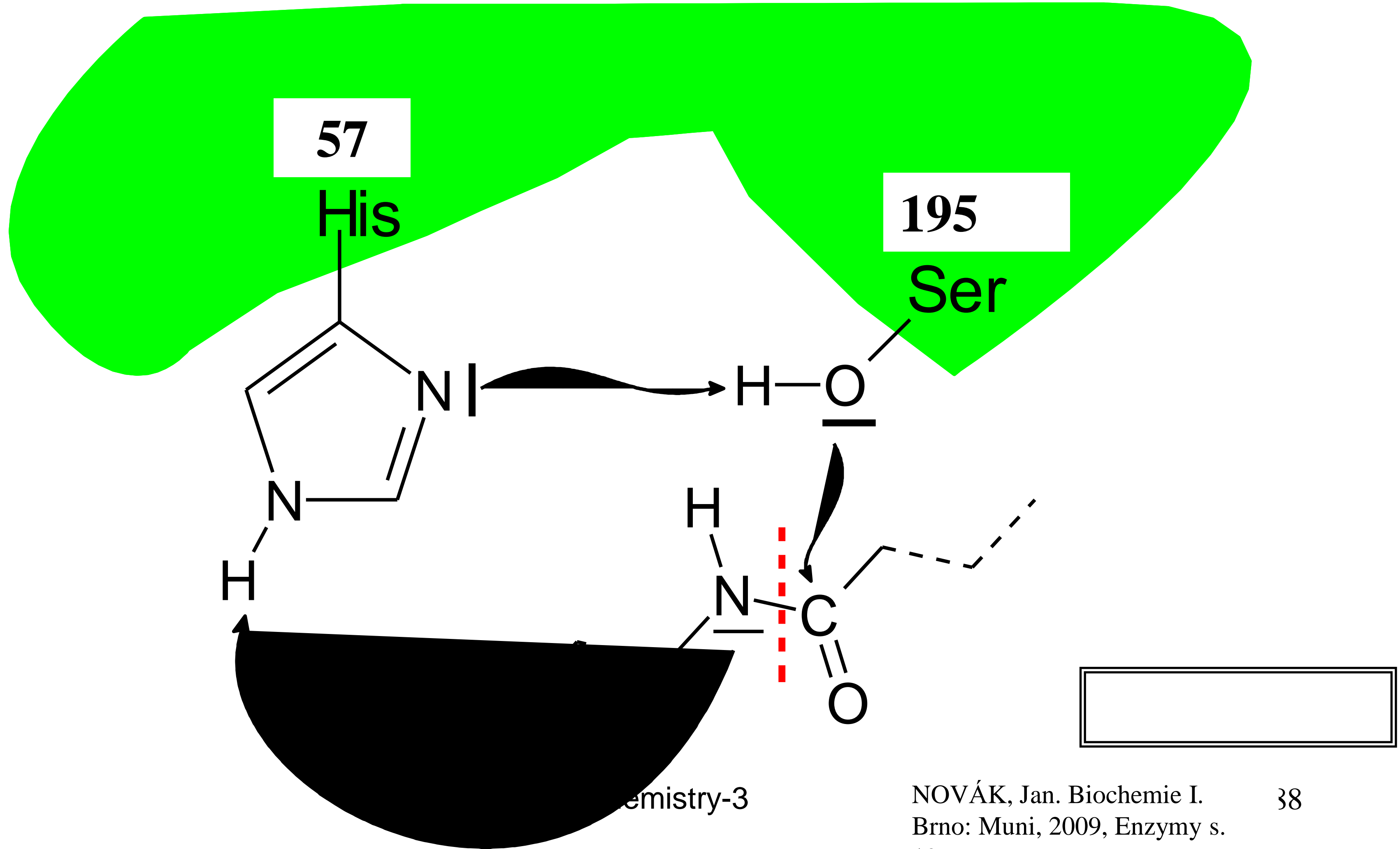
- the realization of chemical transformations in the active site are used so. catalytic groups:
  - nucleophilic (cysteine sulfhydryl, serine OH)
  - acidic (Asp, Glu), - basic (His, Arg, Lys)



- acid-base (proton transfer)
- transient covalent bond
- metal ion catalysis (metalloenzymes)
- electrostatic interactions (without water)
- deformation of the substrate

https://www.youtube.com/watch?v=gJNMryCX3YY

Example: the active site of chymotrypsin? Nucleophilic attack of OH to the carbonyl carbon of serine peptide bond - serine protease



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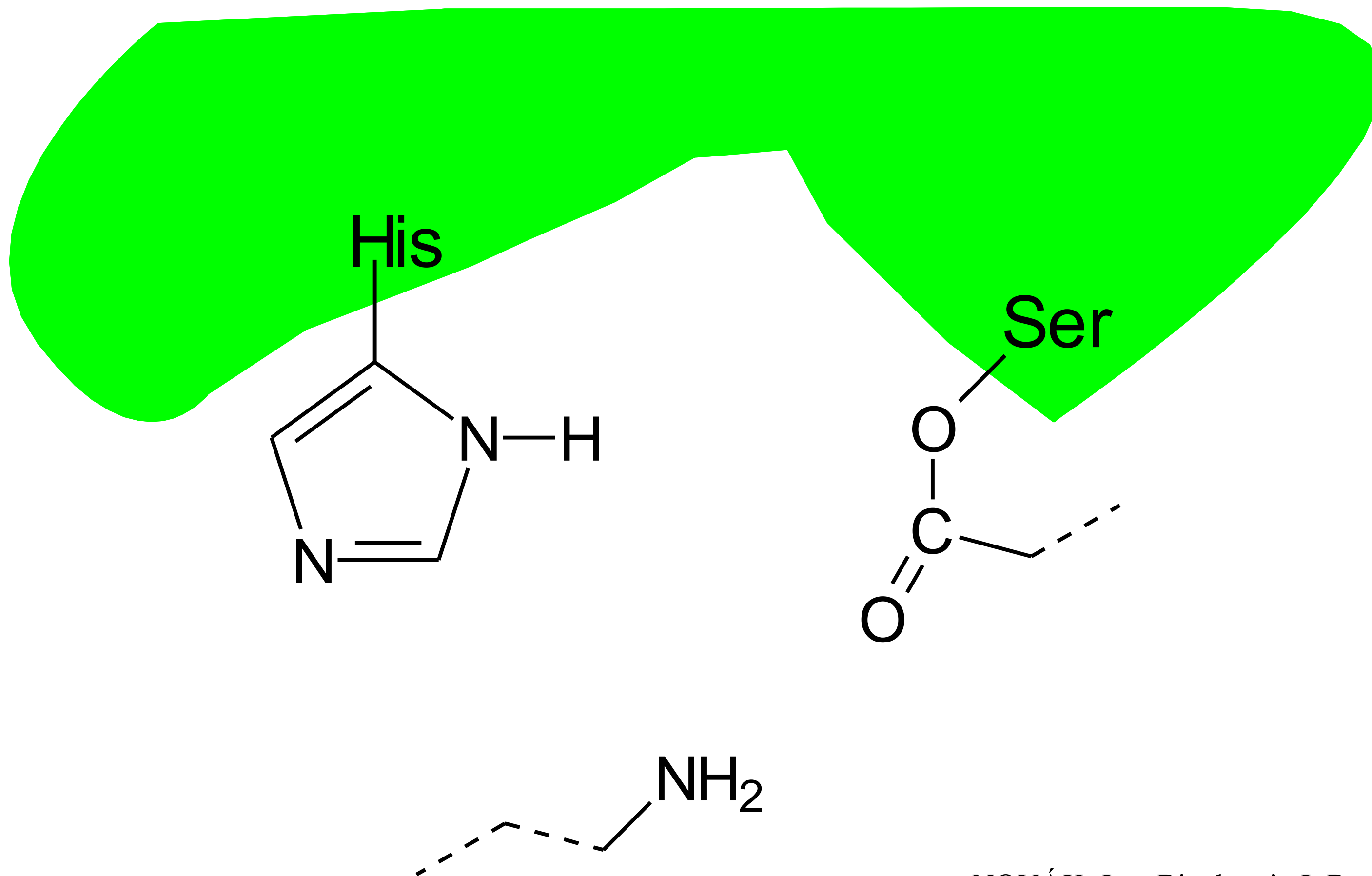
The active site of chymotrypsin: cleavage of peptide bonds



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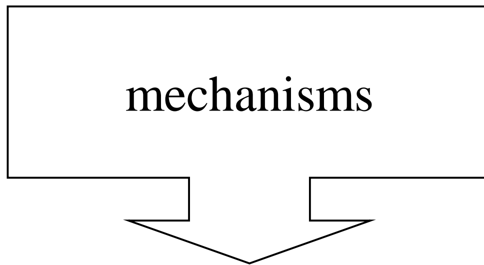
Metaloenzymes

- containing functional metal ions, which participate directly catalyzed reactions, metal ions are bound quite tightly (Enz-M)

  some enzymes need metal ions only to activate, in which case they are bound weakly (Enz ... M), a bivalent metal ions, Ca^{2+} (coagulation factors), Mg^{2+} (kinase)

Metal cation is a part of the ternary complex

- three components form a complex: enzyme (Enz), a substrate (S) and the metal cation (M)
  various types (bridged) complexes Enz-SM, Enz-MS
  sometimes arise cyclic complexes



- to vacant orbitals can accept an electron pair to form a nucleophile-binding
  may form chelates with appropriate groups of the enzyme or substrate structure   deformation tension which facilitates chemical transformation
  coordination sphere of metal acts as a three-dimensional template stereospecific control

molybdenum

- Parts of some oxidoreductases

  Part of the cofactor - molybdopterin

  Xanthine oxidase (xanthine → uric acid)

  Sulfitoxidasa ($HSO_3^- \rightarrow SO_4^{2-}$)

  Aldehydoxidasa (less common, acetaldehyde to acetic acid.)

Sources Mo: legumes, whole grains

Zinc

- Many enzymes
- **Alcoholdehydrogenase** (ethanol → acetaldehyd)
- **carbonatedehydratase** ($H_2O + CO_2 \leftrightarrows H_2CO_3$)
- **carboxypeptidase** (cleavage of the polypeptide from the C-terminus)
- **Cu, Zn-superoxiddismutase** (cytosolic izoform)

  ($2\ {\bullet}O_2^- + 2\ H^+ \rightarrow O_2 + H_2O_2$)

Sources Zn: red meat, shellfish, legumes, sunflower and pumpkin seeds, whole grain cereals

Copper

- oxidoreductases
- **Ceruloplazmin (ferrooxidase)** ($Fe^{2+} \rightarrow Fe^{3+}$)
- **Cytochrom-*c*-oxidase** (RCh, transport of e- to O_2)
- **Monoaminooxidase** (MAO, inactivation of biogenic amines, H_2O_2,
- **Dopaminhydroxylase** (dopamin → noradrenalin)
- **Lysyloxidase** (colagene, Lys → alLys)

Cu Sources: liver, meat, cocoa, legumes, nuts

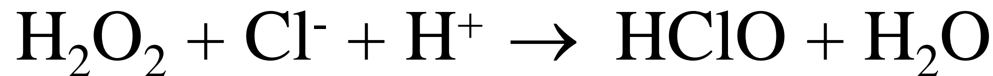
Mangan

- Numerous hydrolases, decarboxylase
  transferase
  Mn-superoxide dismutase (mitochondrial isoform)
- Arginase (($\mathrm{Arg} \rightarrow$ urea + ornithine)
- The synthesis of proteoglycans, glycoproteins

Sources Mn: legumes, whole grains, nuts

Iron

- Heme enzymes, non-heme transporter
- Catalase (hem, $H_2O_2 \rightarrow H_2O + ½O_2$)
- **Myeloperoxidase** (hem, neutrophil)

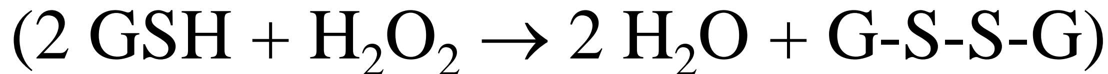


- **Cytochromes (heme electron carrier in the RCh)**
- **Fe-S proteiny** (nehem, přenos elektronů v DŘ)

Sources Fe products from pork, goose, duck blood
(red) meat, liver, egg yolk, nuts

Selen

- Several enzymes (redox reaction), Se is always selenocysteine
- **glutathione peroxidase**



- **Dejodasy thyronin** (thyroxin T4 → trijodthyronin T3)
- **Thioredoxin reductase** (ribose → deoxyribosa)
- **Selenoprotein P** (plasma, antioxidant function?)

Sources Se: cephalopods, marine fish, legumes

Basic concepts of kinetics

- reaction: $S \rightarrow P$ (S = substrate, P = product)
- definition of reaction rate:

$$v = -\frac{\Delta[\mathrm{S}]}{\Delta t} = \frac{\Delta[\mathrm{P}]}{\Delta t} > 0 \quad [\frac{\mathrm{mol}}{\mathrm{l.s}}]$$

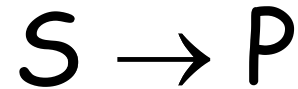
Note: this is defined as the average reaction rate,
instantaneous velocity: d [S] / dt (derivative, share two infinitely small numbers)

What determines the rate of reaction?

- the substrate concentration [S]
  temperature
  in the presence of effector (catalyst inhibitor)

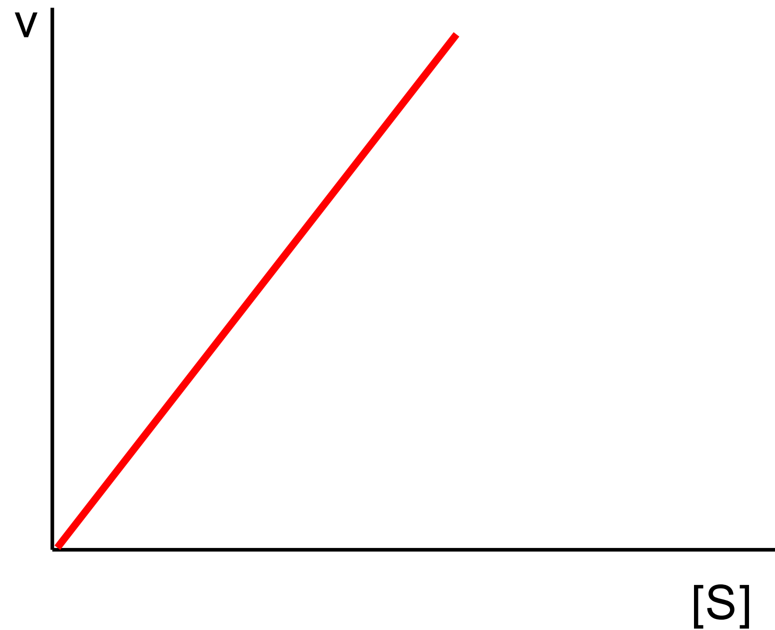
  In addition, enzymatic reactions:
  enzyme concentration [E]
  pH

The kinetic equation for the reaction



$v = k\,[S] = k\,[S]^1 \Rightarrow$ reaction of 1. order

k = rate constant



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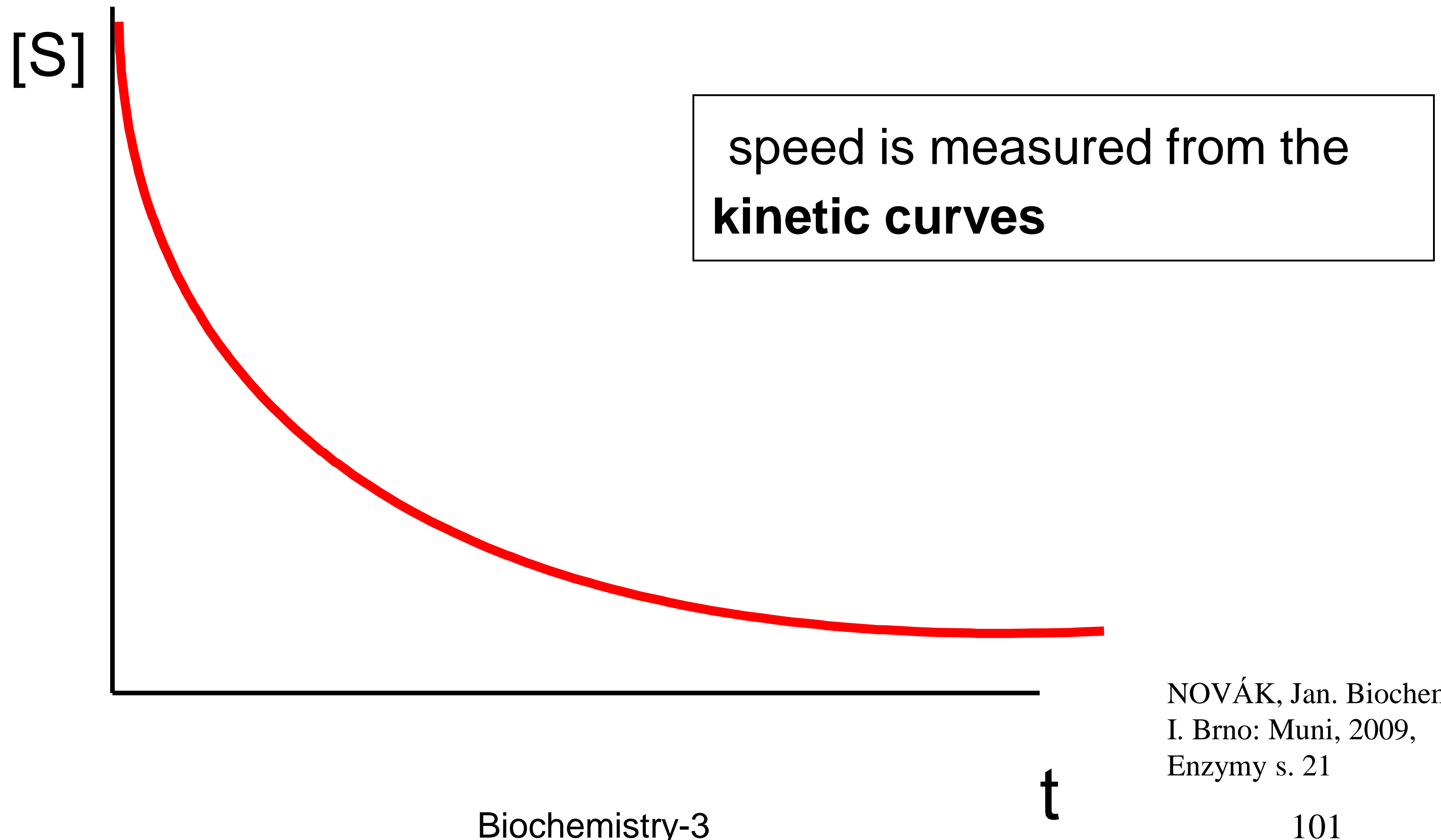
The substrate concentration during the reaction decreases- kinetic curve



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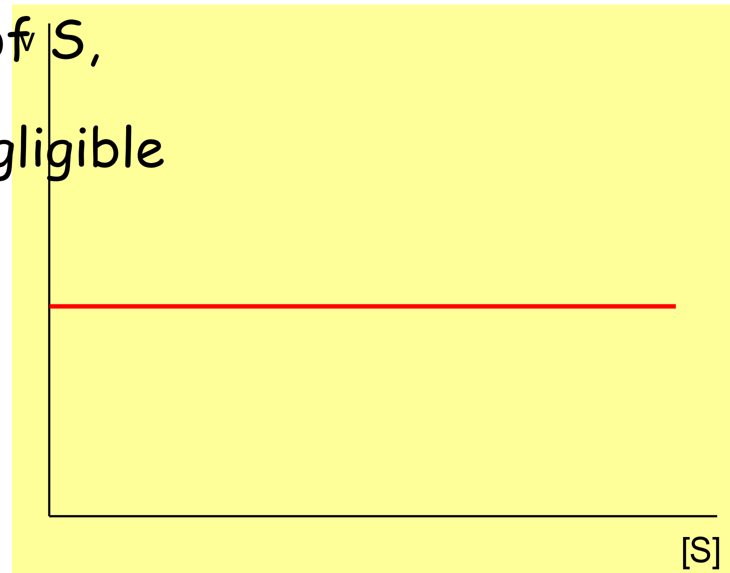
Reaction 0. order is special case

- the reaction rate doesnt depends on the concentration of the substrate
- $v = k\,[S]^0 = k \,.\, 1 = k =$ **constant**
- occurs when a large excess of S,
- so that the loss is almost negligible

on laboratory condition



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The initial rate v_o

- speed measured before significant amounts of product formed
- the highest speed value
- "Virtual value„
- is not affected by the loss of substrate conversion or return the product
- sets of curves,

Dependence of v_o on concetration of substrate

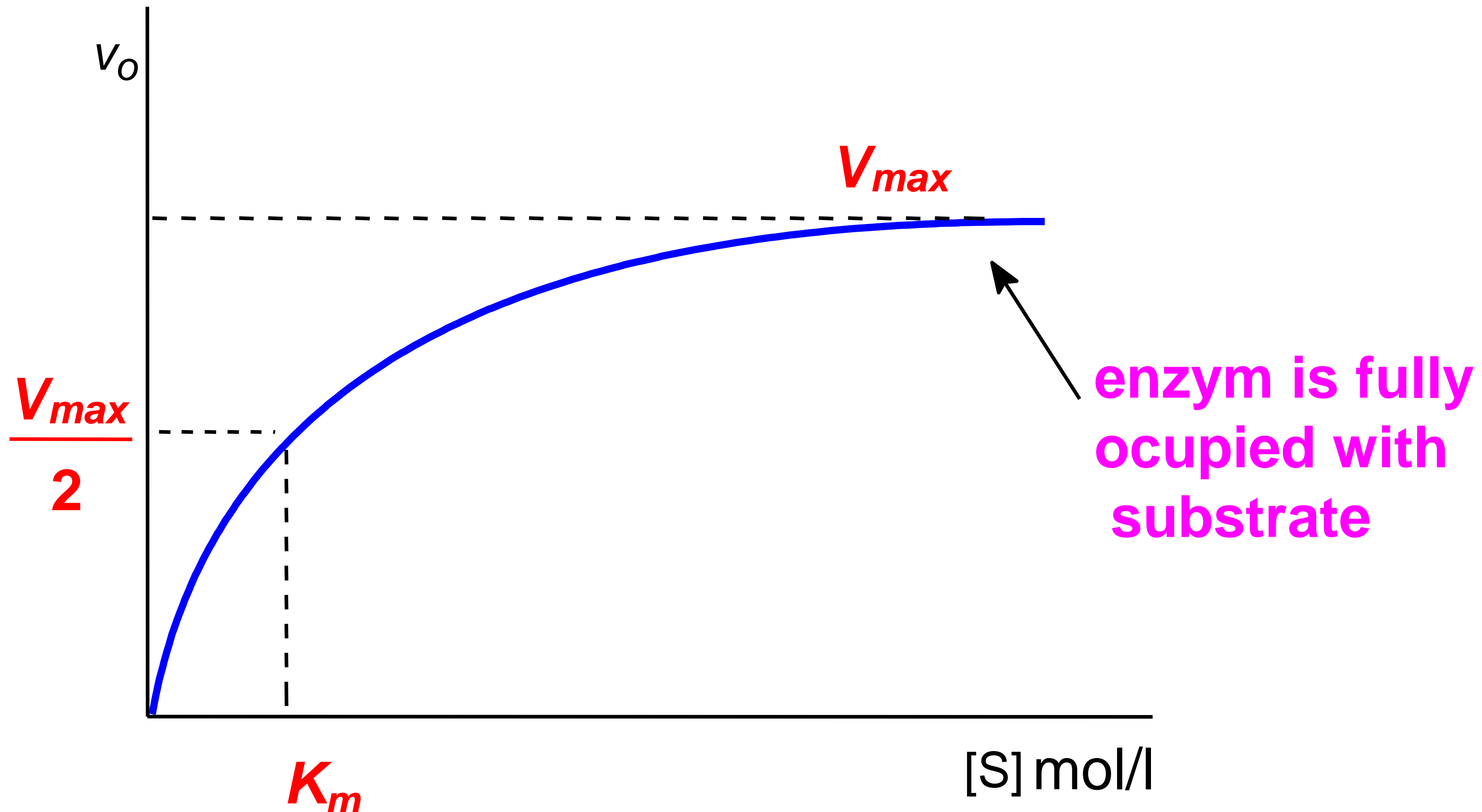
- Michaelis-Menten equation
- single-substrate reaction

$$v_o = V_{max} \frac{[S]}{[S]+K_m}$$

V_{max} = maximal speed (for one concetration of enzyme)

K_m = Michaelis constant

Saturation curve - Michaelis-Menten equation



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For [S] << K_m

$$v_o = V_{max} \frac{[S]}{[S] + K_m} = \frac{V_{max}}{K_m}[S] = k[S]^1$$

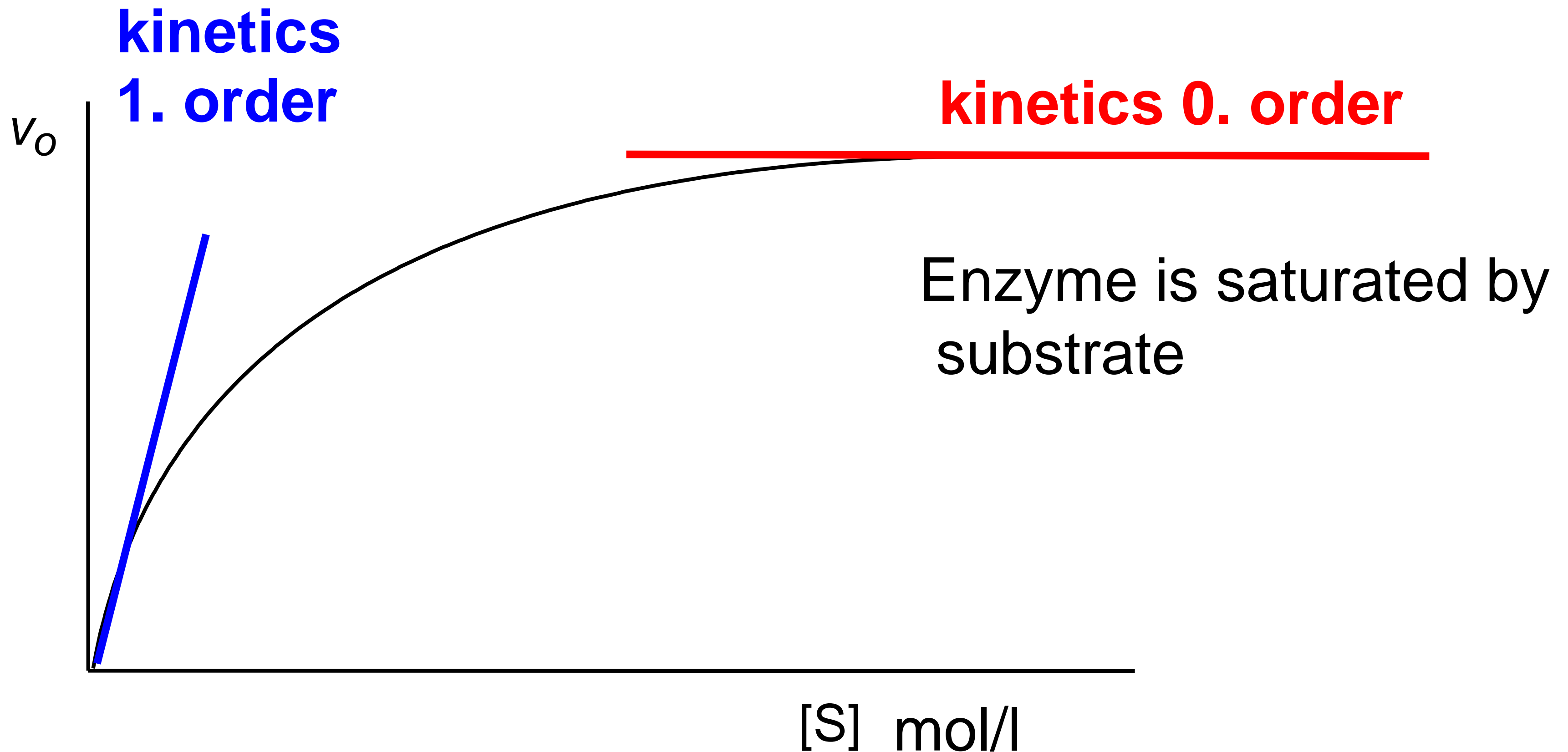
At low substrate concentrations the reaction is governed by 1st order kinetics

$$\text{For } [S] >> K_m$$

$$v_o = V_{max} \frac{[S]}{[S] + K_m} = V_{max} \frac{[S]}{[S]} = V_{max} = k[S]^0$$

At high substrate concentrations, the reaction is governed by the kinetics of the 0th order

Two parts of **saturation curve**



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For $[S] = K_m$

$$v_o = V_{max} \frac{[S]}{[S]+[S]} = V_{max} \frac{[S]}{2[S]} = \frac{V_{max}}{2}$$

Biochemical importance of K_m

- substrate concentration at which the reaction has half of the maximum speed
- at this concentration the enzyme is saturated with 50%
- Km has the dimension of concentration (mol / l)
- Km is inversely proportional to the affinity of the enzyme for the substrate,
- if there is more structurally similar substrates, which has the smallest Km is considered to be the most natural for the enzyme

How to obtain a saturation curve?

- set of experiments, a constant concentration of the enzyme, various concentrations of the substrate ranges from 2 to 3 orders
- from kinetic curvs is estimated v_0
- v_0 is graphically plotted against the relevant [S]
- arises hyperbolic saturation curve

Distinguish

Kinetic curve

- Time record of one reaction
- [S] = f (t)

Saturation curve

- dependence obtained from many of the same reactions
- v_o = f ([S])

[S] …….. concetration of substrate
f …………function
t …… time
v_o ……….. Initial speed

Value V_{max} a K_m characteristics of kinetic properties of enzymes

- is easily determined from the graph of the

  linearized

  Lineweaver-Burk

  double reciprocal plot

- **1/$\boldsymbol{v_o}$** against **1/[S]**

$$v_o = V_{\max} \frac{[\mathrm{S}]}{[\mathrm{S}] + K_m}$$

$$\frac{1}{v_o} = \frac{1}{V_{\max}} \cdot \frac{[\mathrm{S}] + K_m}{[\mathrm{S}]} = \frac{1}{V_{\max}} \left(\frac{[\mathrm{S}]}{[\mathrm{S}]} + \frac{K_m}{[\mathrm{S}]} \right)$$

$$\boxed{\frac{1}{v_o} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}} \cdot \frac{1}{[\mathrm{S}]}}$$

Reciprocal relationship is the equation of a line

$$\frac{1}{v_o} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]}$$

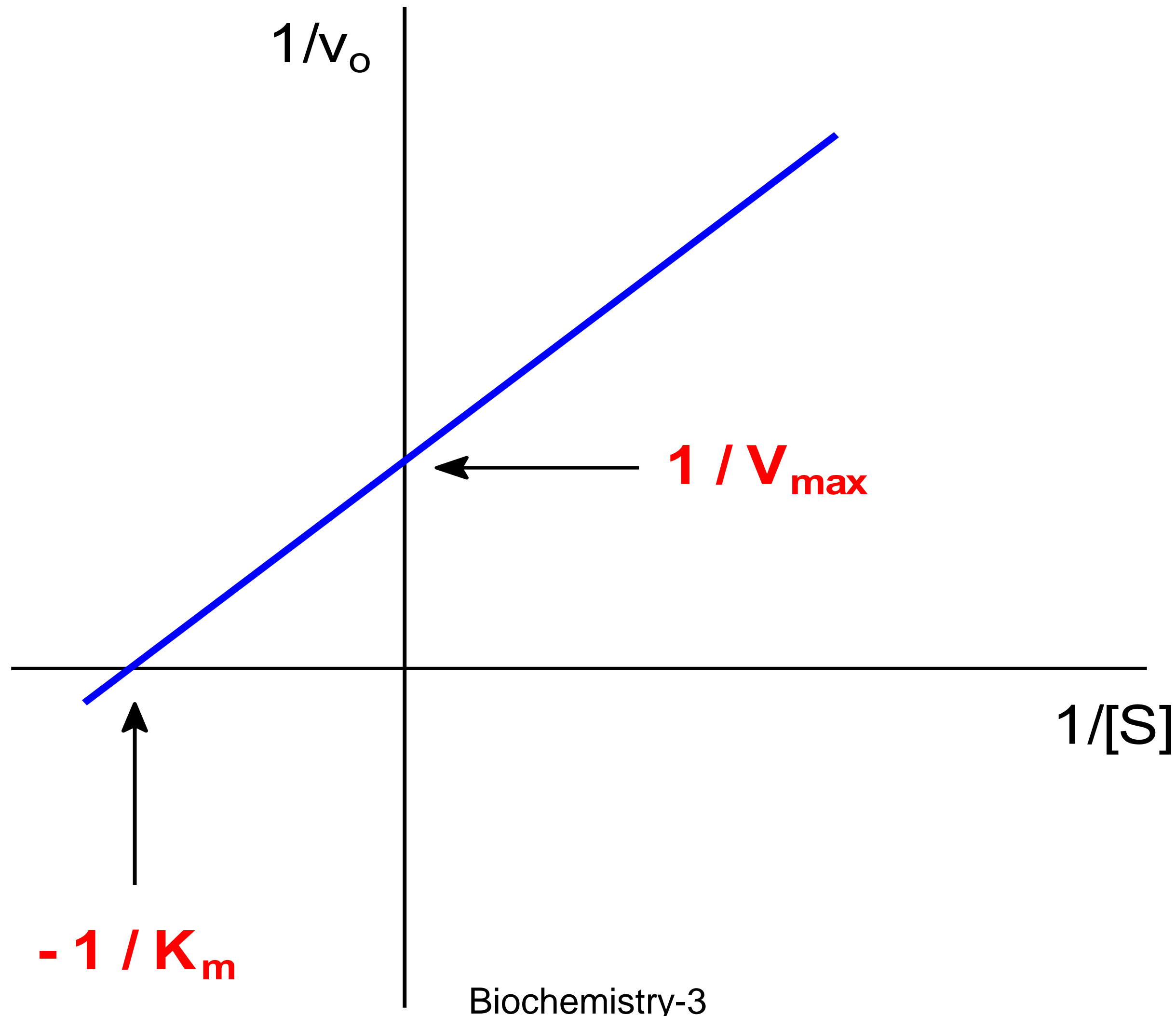
$1/v_o$ .... the dependent variable

$1/[S]$ .... independent variable

K_m/V_{max} .... slope of the line

$1/V_{max}$ ....
the intercept of the dependent variable

Linear graph: $1/v_o$ is function of 1/[S]



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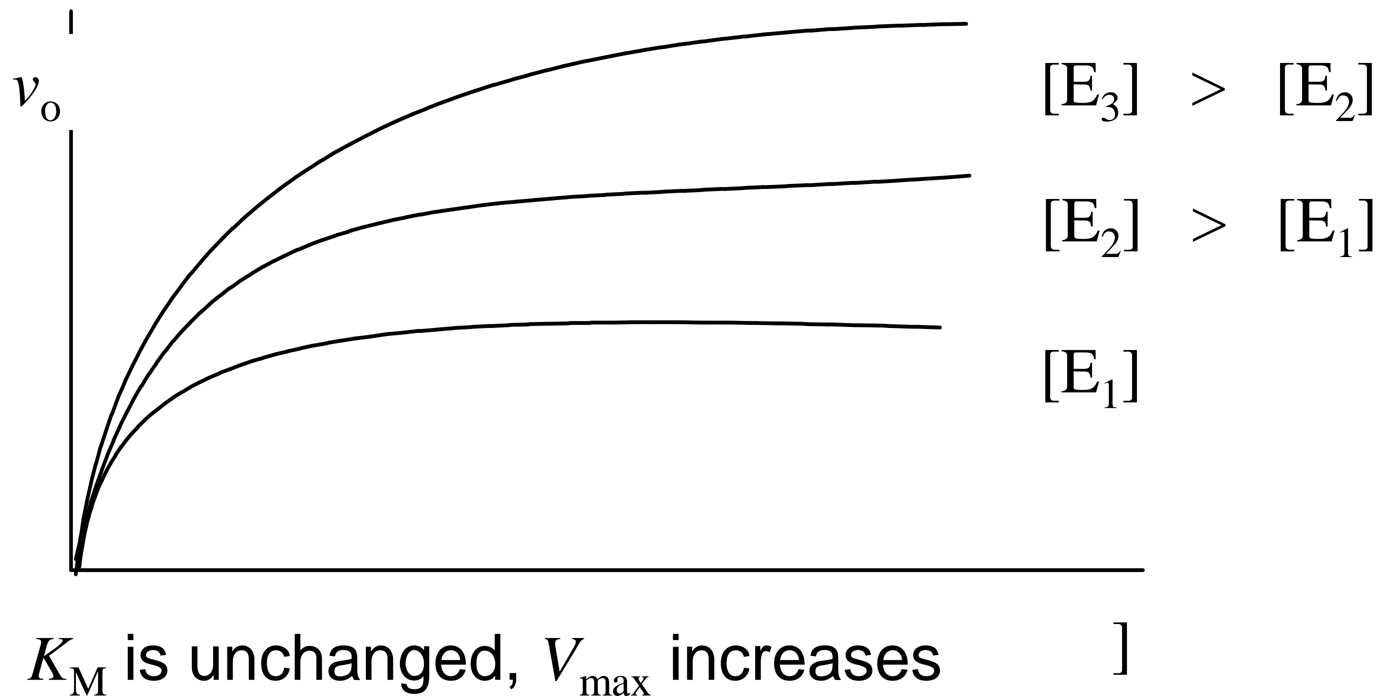
The concentration of enzyme [E] also affects the speed

saturated enzyme : $v_o = k\,[E]_t$

- $[E]_t$ is the total concentration of enzyme

$$[E]_t = [E] + [ES]$$

Saturation curves for different concentrations of enzyme



K_M is unchanged, V_{max} increases

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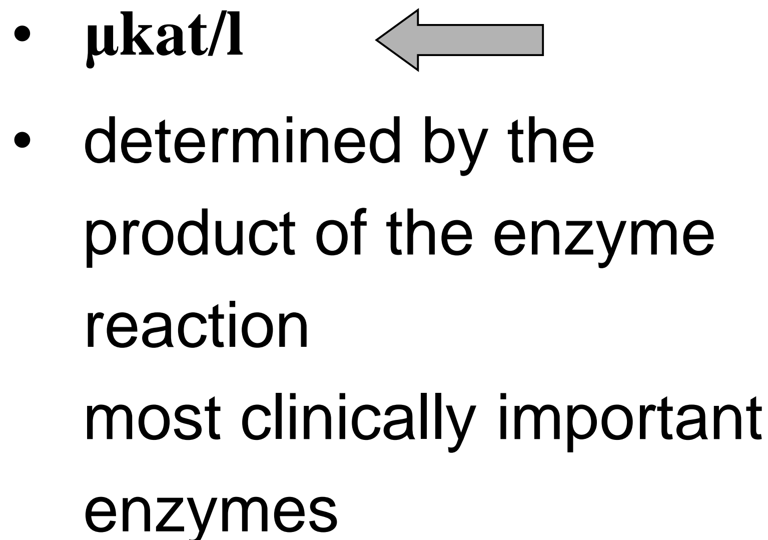
How to determine the amount of enzyme in biological material?

- very difficult
- low (trace) the concentration of enzyme
- present in many other proteins
- normal chemical reactions are not applicable
- not specific for differentiation of individual enzymes

The amount of enzyme in the biological material can be determined in two ways

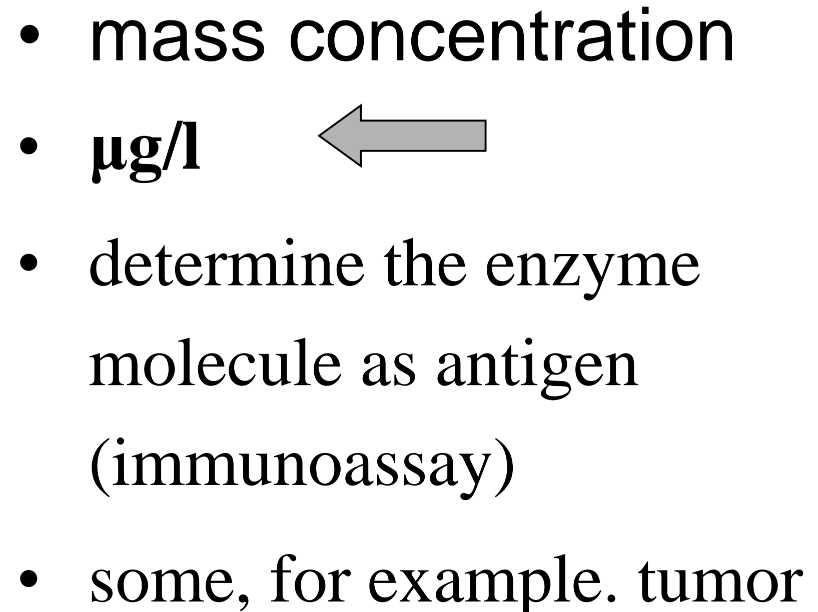
Indirect determination

Catalytic concetration



- **μkat/l**
- determined by the product of the enzyme reaction
  most clinically important enzymes

Direct determination



- mass concentration
- **μg/l**
- determine the enzyme molecule as antigen (immunoassay)
- some, for example. tumor markers

The catalytic activity of the enzyme

- Unit **katal**, 1 kat = mol/s
- One katal is the catalytic activity of the enzyme at which the reaction is converted per mole of substrate for second

IU (international unit)

1 IU = µmol/min

1 µkat = 60 IU

1 IU = 16,6 nkat

Catalytic enzyme concentration

- Activity is based on the volume of biological fluid (blood serum)
- Units mkat/l, μkat/l

Compare the different analytical approaches

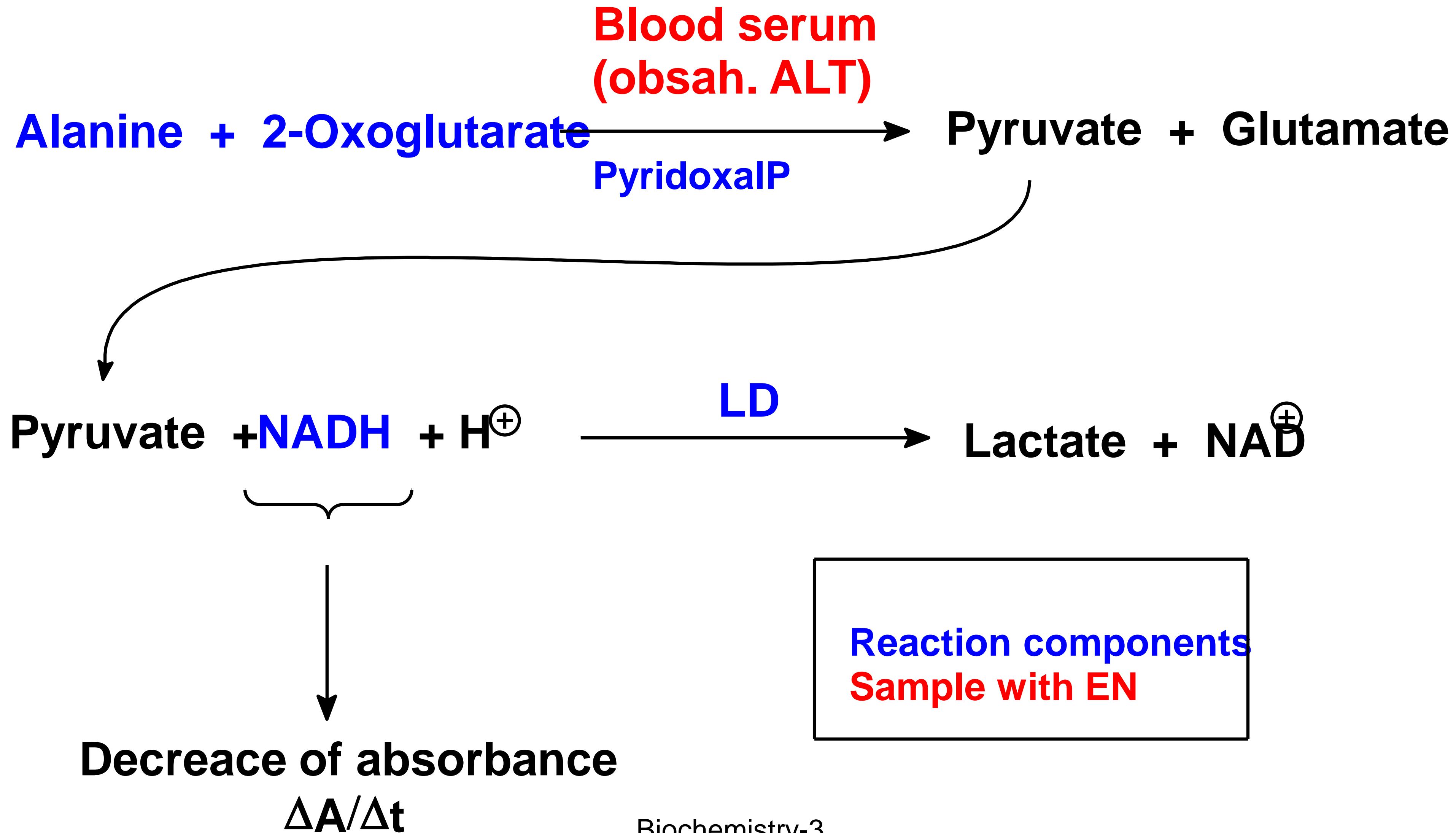
Glucose

- substrate
- low molecular weight concentration in serum
- 3,3-5,6 **mmol/l**
- Glucose is determined directly

ALT

- enzyme
  high molecular weight
  cat. concentration in serum
- 0,2-0,9 **μkat/l**
- determined not by the enzyme, but the product or a cofactor in enzymatic reactions

Methodology for determination of ALT (all ingredients are colorless)



Determination of the catalytic activity in laboratory

- optimal conditions (temperature, pH, cofactors)
- measured Δ[S] or Δ[P] in a certain time interval
- kinetics of the 0th order, [S] >> Km -----
- saturated enzyme, velocity is constant,
- Approaching V_{max}

Two methods for the determination of catalytic concentration of enzymes

- *Kinetic*
- continuously measured [S] or [P] (eg., after 10 s)
- Plot the kinetic curve it is found in the kinetic curves
- accurate method

constant time/End point

- measured [P] after the reaction
- kin. curve is not needed
- average speed
- Δ[P]/Δ t
- Less accurate method

Enzyme inhibition (reduction of activity)

irreversible

inhibitor tightly bound to the enzyme (active place)

organophosphates

heavy metal ions

cyanides

reversible

loosely bound inhibitor

balance E + I .... E-I

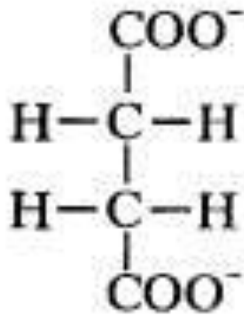
inhibitor can be removed (dialysis, gel. filtration)

two basic types: competitive, noncompetitive

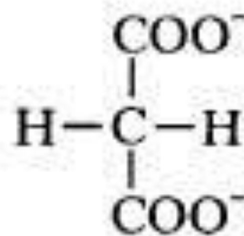
competitive inhibition

- inhibitor is structurally similar to the substrate
- binds to the active site
- competes with the natural substrate binding site

Natural substrae x **competitive inhibitor**



Succinate

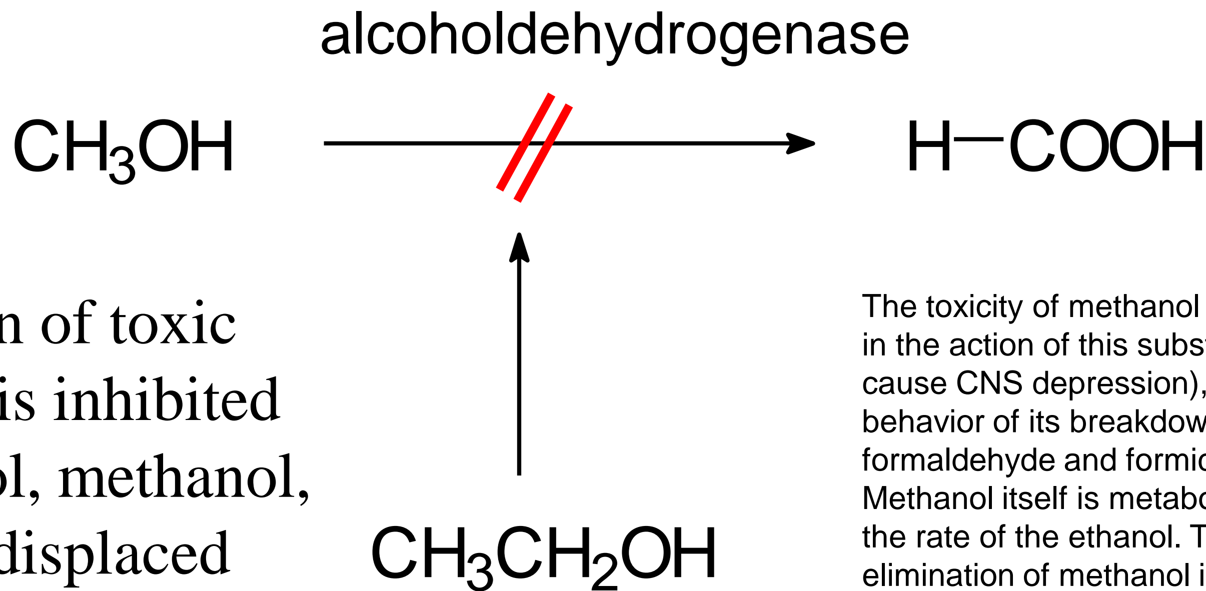


Malonate

Malonate is inhibitor of succinatedehydrogenase

http://www.s-cool.co.uk/assets/test_its/alevel/biology/biological-molecules-and-enzymes/quest24.jpg

Methanol poisoning is treated with ethanol

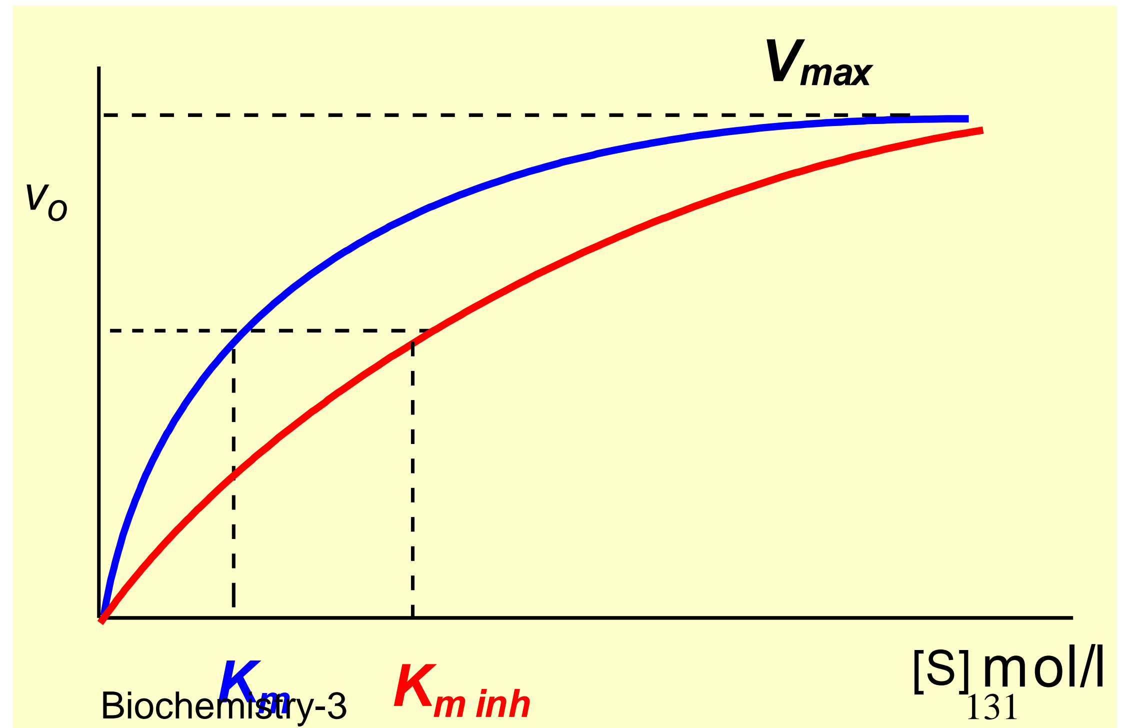


Formation of toxic products is inhibited by ethanol, methanol, which is displaced from the binding site of the enzyme - competitive inhibition dehydrogenation of methanol

The toxicity of methanol lies not so much in the action of this substance (even cause CNS depression), but rather in the behavior of its breakdown products - formaldehyde and formic acid. [11] Methanol itself is metabolized roughly half the rate of the ethanol. The total elimination of methanol is slow, corresponding to roughly one-seventh the speed for ethanol. Moreover, ethanol has about twenty times higher than the affinity for alcohol dehydrogenase methanol, therefore, the preferred substrate. This allows administered ethanol (also fomepizole) as antidote for poisoning because it significantly slows down the metabolism of methanol, and thus significantly reduce its biochemical and clinical effects.

Competitive inhibition

- maximum speed is reached at higher value [*S*]
- V_{max} no change
- K_m increase



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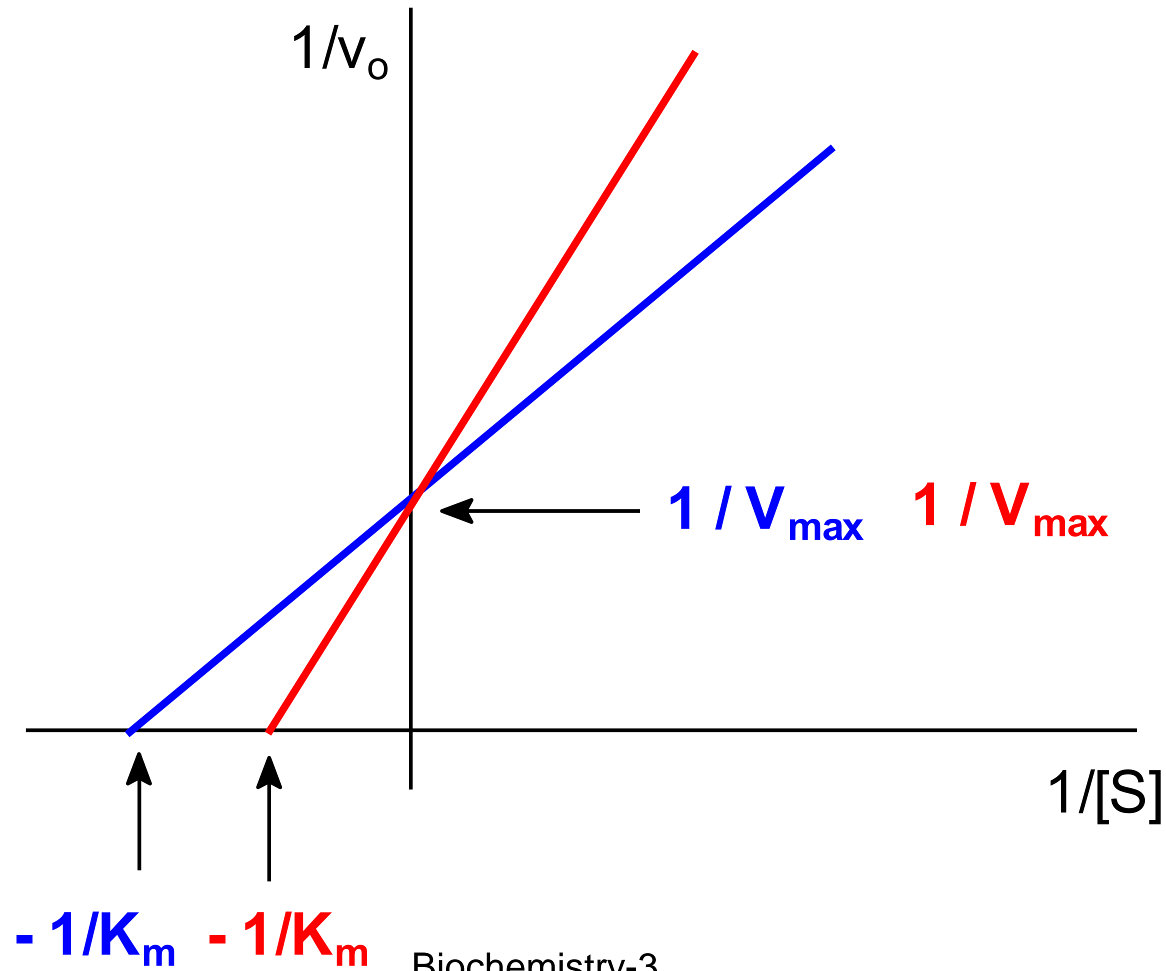
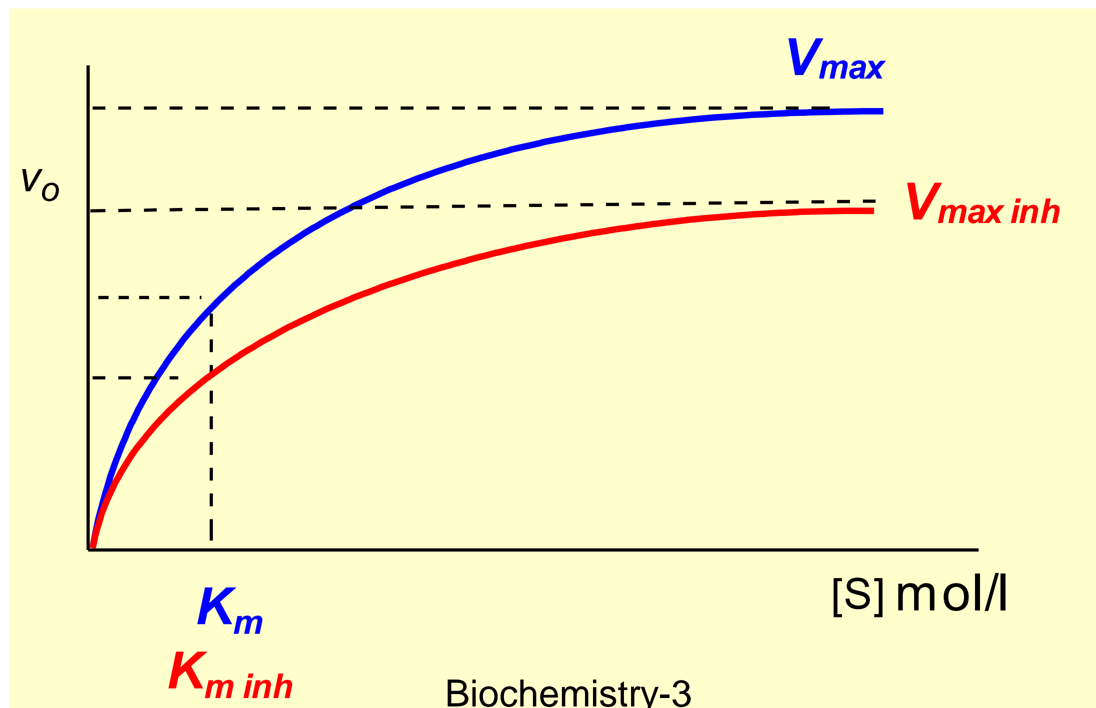
Competitive inhibition



Noncompetition inhibition

- The inhibitor binds outside the active center on the complex E and ES
- Km does not change (the active site is free for substrate)
- Vmax is reduced because the concentration decreases functional complex



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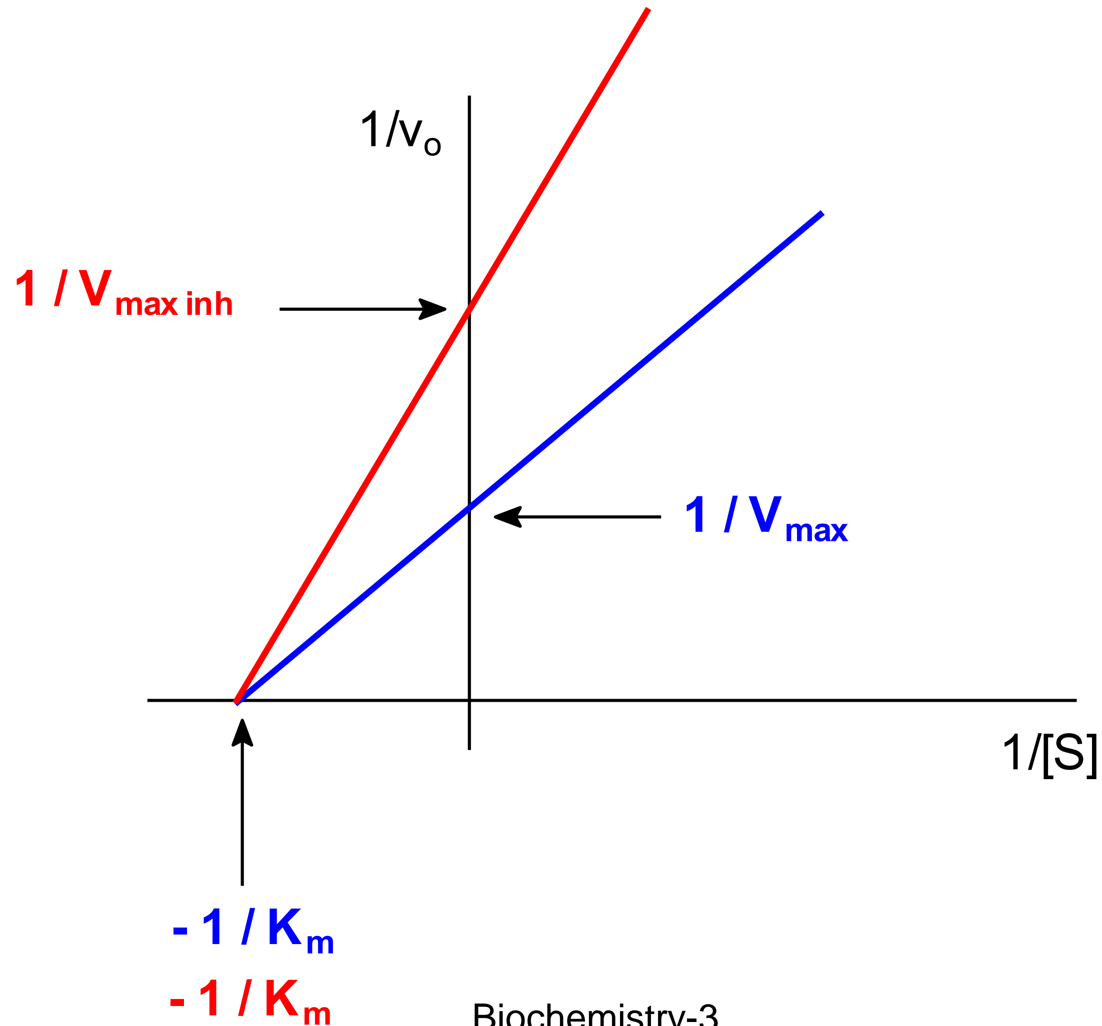
Non competitive inhibition



Many drugs are enzyme inhibitors

- Acetylsalicylic acid (cyclooxygenase)
- Ibuprofen (cyclooxygenase)
- Statins (HMG-CoA reductase) - lipid lowering drugs reduce cholesterol synthesis (lovastatin)
- ACE inhibitors (angiotensin converting enzyme) - treatment of hypertension (enalapril)
- Reversible inhibitor of acetylcholinesterase (neostigmine) - neuromuscular disease, postoperative intestinal atony
- Selective brain acetylcholinesterase inhibitors (rivastigmine, galantamine) - Alzheimer's disease

Antibiotics inhibit the enzymes necessary for a life of bacteria

- Penicillin - inhibit transpeptidasy (construction of cell walls)
- Tetracyclines, macrolides, chloramphenicol - inhibition of protein synthesis
- Fluorinated quinolones (ciprofloxacin) - inhibition of bacterial gyrase (topoisomerase II) (untwisting of DNA during replication)

Regulation of enzyme activity (three general ways)

1. Control of the number of molecules of the enzyme
2. Regulation of the biological activity of the enzyme
3. Availability and concentration of substrate and / or cofactor (in vivo less significant factor)

Regulating the amount of enzyme

- Controlled constitutive enzyme, protein synthesis and induce the expression of genes, speed regulation of transcription, post-transcriptional modifications of RNA, regulation of the speed of translation and posttranslational modifications
- Controlled degradation enzyme specific intracellular proteases - determine the different biological half-lives of enzymes

Regulation of the biological activity of the enzyme

- **Isoenzymes** (one type of response is regulated differently in various tissues)
- Activation of the enzyme and irreversible partial proteolysis
- Breakage of covalent modification of the enzyme
- allosteric regulation

Isoenzymes

- catalyze the same reaction, but differ in primary structure and thus phys.-chem. and kinetic properties
- often have different tissue distribution
- They are determined by electrophoresis
- isoforms - a more general term (include more pseudoizoenzymy, posttranslational variants)

Creatinkinase (CK) is dimer and form isoenzymes

Izoenzyme		**% of activity**	**increased**
CK-MM	muscle	94-96%	muscle trauma
CK-MB	heart	to 6%	heart attack
CK-BB	Brain	trace	brain Injury

In the cells, the "cytosolic" CK enzymes consist of two subunits, which can be either *B* (brain type) or *M* (muscle type). There are, therefore, three different isoenzymes: CK-MM, CK-BB and CK-MB. The genes for these subunits are located on different chromosomes: *B* on 14q32 and *M* on 19q13. In addition to those three *cytosolic* CK isoforms, there are two mitochondrial creatine kinase isoenzymes, the *ubiquitous* and *sarcomeric* form. The functional entity of the latter two mitochondrial CK isoforms is an octamer consisting of four dimers each.[4]
While mitochondrial creatine kinase is directly involved in formation of phospho-creatine from mitochondrial ATP, cytosolic CK regenerates ATP from ADP, using PCr. This happens at intracellular sites where ATP is used in the cell, with CK acting as an *in situ* ATP regenerator.

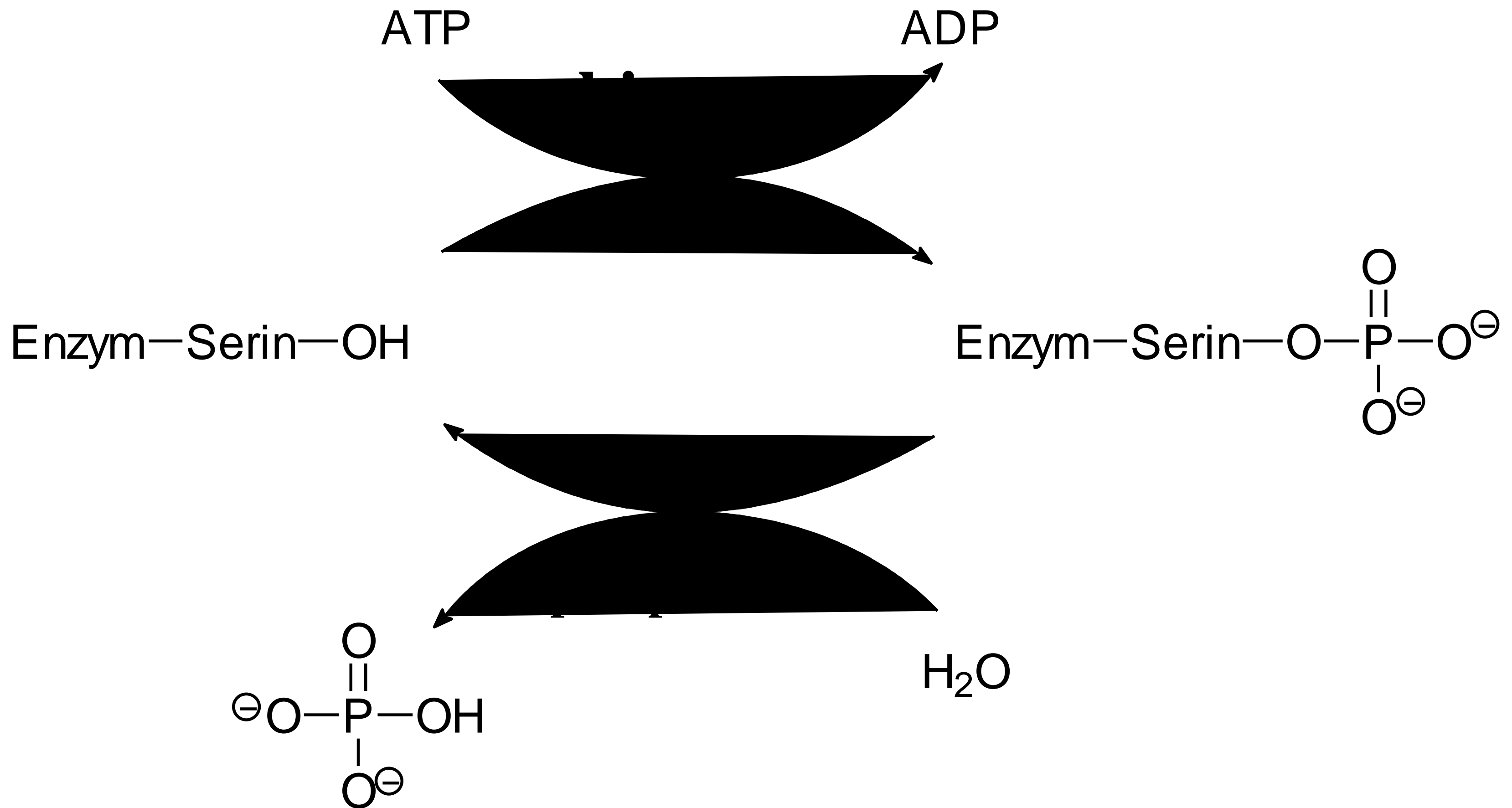
Activation of the enzyme by partial proteolysis

- Active enzyme arises irreversible cleavage of a particular sequence of the proenzyme molecule
- Proteinase in GIT (pepsinogen pepsin)
- Blood clotting factors
- Proteases (caspases) activated during apoptosis

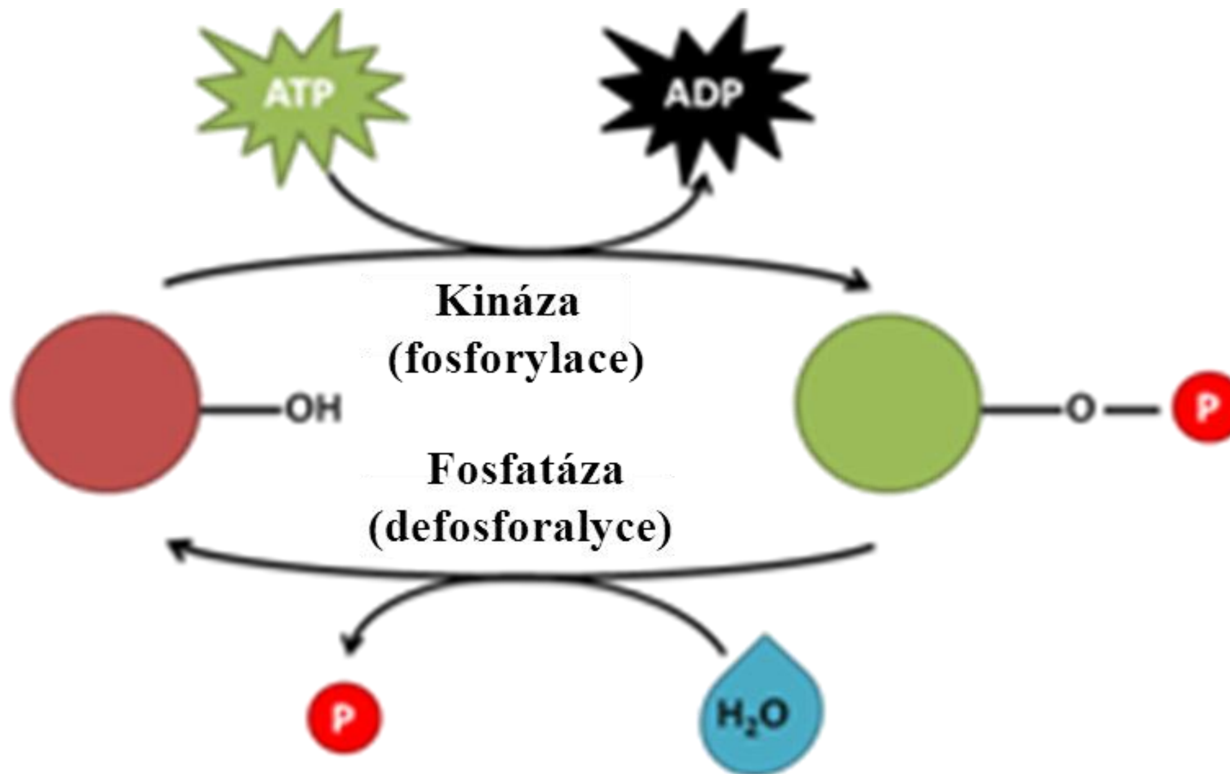
Reversible covalent modification of the enzyme

- phosphorylation catalyze kinase -PO3 2- phosphoryl transfer from ATP to the OH group of the enzyme (Ser, Thr, Tyr)
- reciprocal plot (dephosphorylation) phosphatase catalyze the hydrolysis of ester-bound phosphate
- Other modification: carboxylation, acetylation………………

Phosphorylation and dephosphorylation of enzyme



Mechanism of phosphorylation



http://web2.mendelu.cz/af_291_projekty2/vseo/stranka.php?kod=1722

Examples

glycogen phosphorylase

Catalyzes the cleavage of glycogen by inorganic. Phosphate

Phosphorylated enzyme is active

Dephosphorylated enzyme is inactive

Glykogensynthasa

Catalyzes the synthesis of glycogen from UDP-glucose

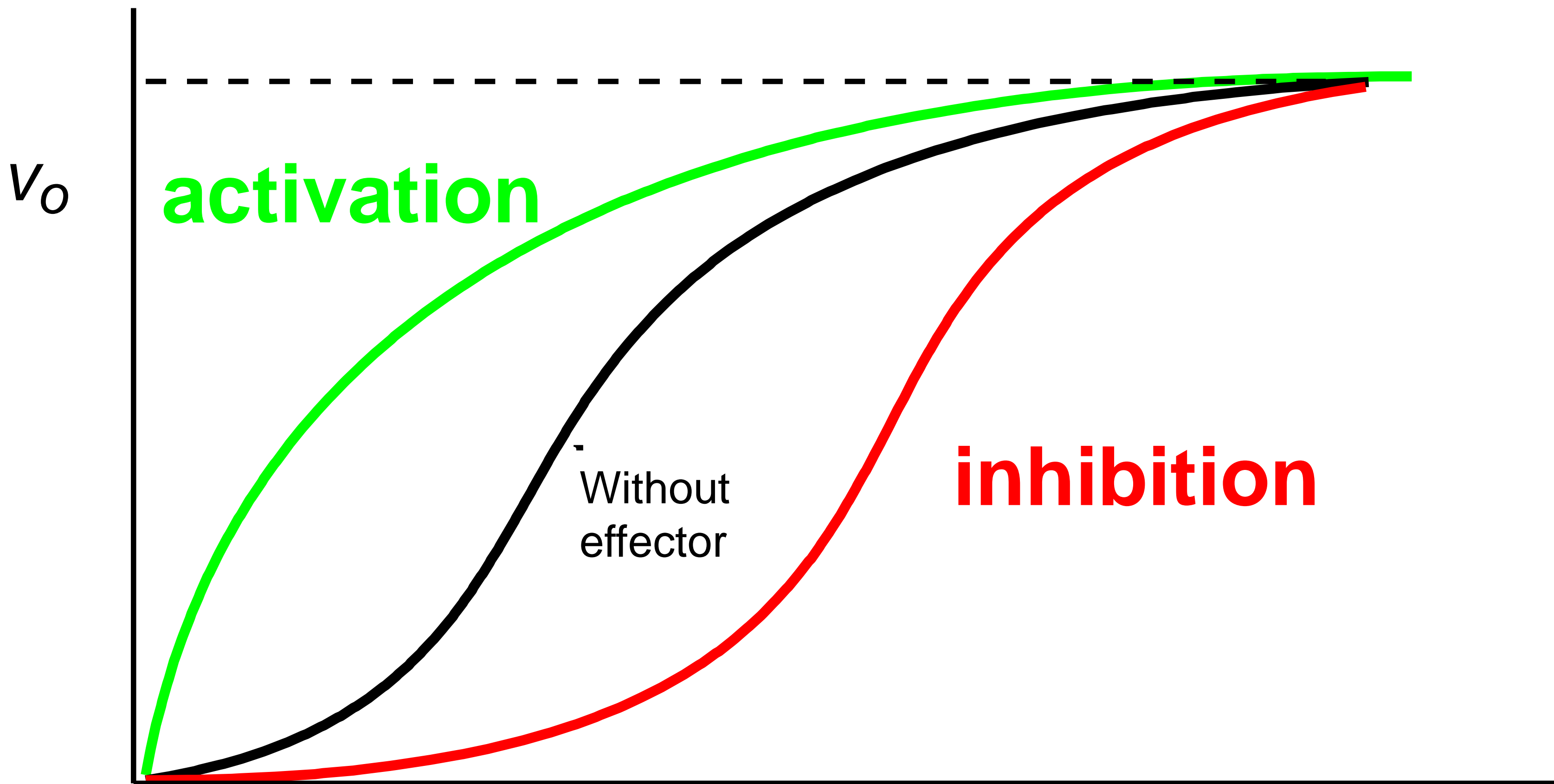
The phosphorylated enzyme is inactive

Dephosphorylated enzyme is active

Allosteric enzymes are oligomeric

- multiple subunits often regulatory and catalytic
- the enzyme binds effector structurally distinct from the substrate often product
- binds to the allosteric sites - other than the active site
- binding causes a conformational change in the enzyme activity …. change - allosteric activation or inhibition

Saturation curve of allosteric enzymes are sigmoid



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Cooperative effect

- for oligomeric enzymes and proteins
- more subunits --- more binding sites
- binding of substrate (or other substance) to one subunit induces a conformational change in the other, that bind other molecules more easily (difficult)
- Example: hemoglobin × myoglobin

Using of enzymes in medicine

1. enzymes as indicators of a pathological condition: when cell damage increases intracellular enzyme activity in the extracellular fluid
2. enzymes as analytical reagents in her lap. biochemistry
3. enzymes as medicaments

Examples of enzymes in clinical diagnosis

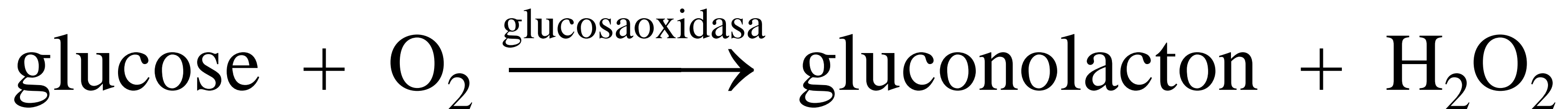
Enzym e	Referenční hodnoty	Interpretace zvýšení
ALT (alaninaminotransferase)	to 0,9 μkat/l	hepatopatie
CK (creatinkinase)	to 4 μkat/l	myopatie, heart atact
PSA (prostatic specific antigene)	to 4 μg/l	carcinom of prostate

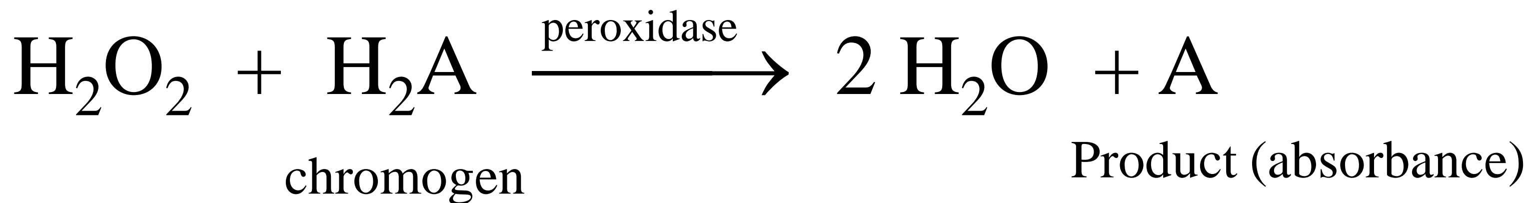
[a] The serum values for men over 15 years
ALT alanine aminotransferase, creatine kinase CK,
PSA prostate specific antigen, neuron specific enolase NSE

Enzymes as analytical reagents

Enzyme	**Origine of enzyme**	The substance / method
Glucosaoxidase	*Aspergillus niger*	glucose
Peroxidase	křen (*Armoracia* sp.)	glucose
Lipase	*Candida* sp.	triacylglycerol
Cholesteroloxidase	*Pseudomonas* sp.	cholesterol
Uricase	*Candida* sp.	Uric acid
Bilirubinoxidase	*Myrothecium* sp.	bilirubin
Urease	bob (*Canavalia* sp.)	urea
Lactatdehydrogenasa	*Pediocus* sp.	ALT, AST
***Taq* polymerase**	*Thermus aquaticus*	PCR

Enzymatic determination of glucose





Principle of glucose analyzers

Personal glucometers

- intended for personal control diabetic
- glucose oxidase is anchored on the solid phase
- H_2O_2 produced is determined by another method (using Pt electrodes)
- the display shows the concentration of glucose (mmol / l)

Personal glucometers

http://zdrav-pro.cz/wp-content/uploads/glukometr-g423-1.2.jpg

Pancreatic enzymes in therapy

- a mixture of enzymes (lipases, amylases, proteases) obtained from porcine pancreas
- indication: pancreatic secretory insufficiency of different etiology, cystic fibrosis
- dosage: 3 times a day with meals
- range of OTC products

acid-resistant capsules to disintegrate in the duodenum

Asparaginase in the treatment of leukemia

- Catalyze hydrolysis of the amide group asparagine
- $Asn + H_2O \rightarrow Asp + NH_3$
- L-asparagine is essential for protein synthesis of some tumor cells
- Hydrolysis of Asp leads to a reduction of proliferation
- Indications: acute lymphoblastic leukemiaKatalyzuje hydrolýzu amidové skupiny asparaginu

fibrinolytic enzyme

- drugs that dissolve blood clots
- streptokinase (bacterial), urokinase (human)
- cleaves plasminogen to plasmin - that causes degradation of fibrin and thrombolysis
- indications: venous thrombosis, pulmonary embolism, acute IM

Proteases in local therapy

- fibrinolysin, chymotrypsin, collagenase
- after topical application leads to lysis of necrotic tissue, do not harm healthy cells (containing protease inhibitors)
- main indication in surgery
- festering wounds, venous ulcers, diabetic gangrene, pressure ulcers, postoperative wounds, etc..

Proteases in systemic therapy (oral administration)

- Trypsin, chymotrypsin, plant proteases - papain (papaya), bromelain (pineapple)
- Some studies suggest an anti-inflammatory effect, influencing immunity in autoimmune disease
- Indication: auxiliary drug for rheumatoid arthritis, traumatic inflammation and edema, lymphoedema, phlebitis, etc..
- CHC, quite expensive (Wobenzym, Phlogenzym etc..) to 30 tabl. daily

Example 1

When the enzymatic reaction of a substrate solution was added to the buffer containing the enzyme is added to the sample (0.1 ml). After 5 min was determined 0.2 mmol of product.
What is the catalytic concentration of enzyme in the sample?

Example 1 - Solution

t = 5 min = 5. 60 s = 300 s

for 300 s … 0.2 mmol of product formed

for 1 s … x = 0,2/300 = 6,7 .10^{-4} mmol / 0,1 ml sample

for 1 litr sample = 6,7 . 10^{-4} . 10^{4} = 6,7 mmol/l.s = **6,7 mkat/l**

Example 2

The reaction mixture contained:

2.5 ml buffer

0.2 ml of coenzyme NADH (optical test)

0.1 ml of blood serum

0.2 ml of substrate solution

For 60 s was a decrease in absorbance of coenzyme $\Delta A = 0{,}03$. $\varepsilon_{NADH} = 6220$ l/mol.cm, L = cuvette width of 1 cm. What is catalytic concentration of enzymes?

Solution of example 2

Serum was diluted : V_{con}/V_{orig} = 3,0 / 0,1 = 30

Lambert-Beer: $\mathbf{\Delta A = \varepsilon \Delta c\, l}$ / for $\Delta t \Rightarrow$

60 s:

$$\frac{\Delta c}{\Delta t} = \frac{\Delta A}{\varepsilon \cdot l \cdot \Delta t} = \frac{0{,}03}{6220 \cdot 1 \cdot 60} = 8.10^{-8} \text{ mol/l.s}$$

Dilution: $30 . 8 .10^{-8} = 2{,}4 . 10^{-6}$ mol/l.s =

$2{,}4 . 10^{-6}$ kat/l = **2,4 μkat/l**

catalytic enzyme concentration = chemical reaction rate

[mol / l.s]