ENZYMES

= substances that ..... biological reactions

1. Provide an alternative reaction route which has a lower ..... energy
2. Reactions catalysed by enzymes occur under mild conditions + good yield + fast
3. Enzymes are specific – catalyse the reactions of one substance only or of a very limited range of substances

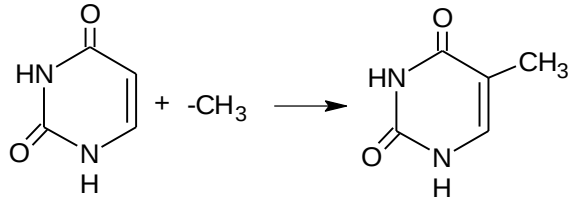
SUBSTRATE = a substance which an enzyme enables to react.

Classification of enzymes:

- **Oxidoreductases** – most common, catalyse reactions connected with an ..... transfer, hydrogen transfer or reactions with .....

  e.g. ethanol + NAD^+ → acetaldehyde + NADH + H^+ is catalysed by alcoholdehydrogenase

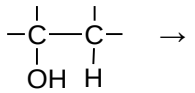
- **Transferases** – enable the transfer of groups (-CH_3, -NH_2, P_i(phosphate ion))

e.g. 

uracil thymine

- **Hydrolases** – enable hydrolytic breakdown (fission), e.g.:
  - $C_{12}H_{22}O_{11}$ + H_2O → $C_6H_{12}O_6$ + $C_6H_{12}O_6$ catalysed by .....
    sucrose ....
  - starch + H_2O → maltose catalysed by .....
  - proteins + H_2O → aminoacids, peptides catalysed by ....., e.g. .....

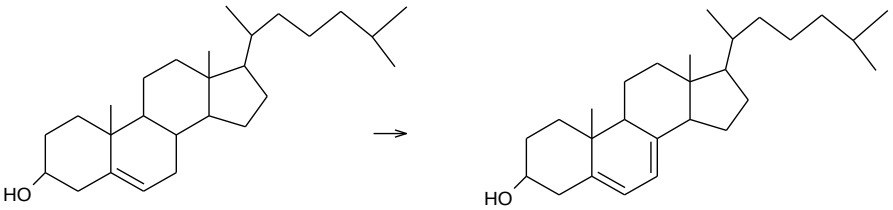
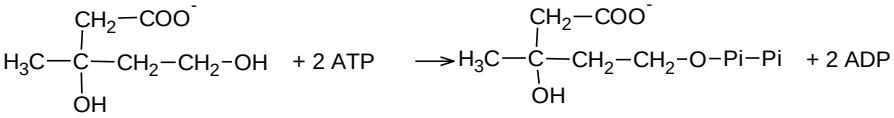
- **Lyases** – catalyse fissions without water, elimination, leads to the formation of a double bond.

- dehydratases:  →
- decarboxylases: HOOC−COOH →

- **Isomerases** – catalyse intramolecular changes

- **Ligases –** catalyse bond formations connected with the breakdown of energy rich phosphate bonds.

  $X + Y + ATP \rightarrow X{-}Y + ADP + P_i$

1. *What types of enzymes catalyse the following reactions?*
   a. $^{-}OOC{-}CH_2{-}CO{-}COO^{-} \rightarrow CH_3{-}CO{-}COO^{-}$
   b. $CH_2{=}C(CH_3){-}CH_2{-}CH_2{-}O{-}P_i{-}P_i \rightarrow CH_3{-}C(CH_3){=}CH{-}CH_2{-}O{-}P_i{-}P_i$
   c. *lipids + H_2O → glycerol + fatty acids*
   d. 
   e. 

Structure of an enzyme molecule

Enzymes = mostly proteins

Some enzymes (HOLOENZYMES) consist of a protein part (APOENZYME) and a non-protein part (COFACTOR)

HOLOENZYME (catalytically active complex) = APOENZYME + COFACTOR

Cofactors:

- Metal ions in metaloenzymes
  - Components of the active site of the enzyme

- Bridges for bonding the substrate
- Stabilise the structure of the enzyme

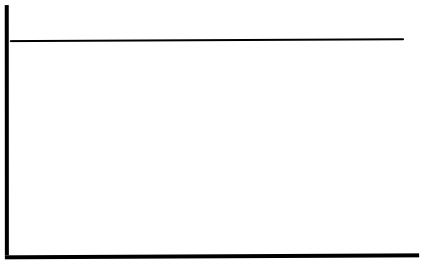
- Organic molecule :
  COENZYMES = carriers of functional groups, atoms or electrons in the reactions they catalyse, bonded by a weak force to the enzyme
  PROSTHETIC GROUP = permanently bonded to the enzyme
  Some coenzymes:
  - NAD^+ (nicotinamide adenine dinucleotide) – derived from nicotinic acid (vitamin PP)
  - $NADP^+$ (phosphate of NAD^+)
  - FAD (flavin adenine dinucleotide) – derived from riboflavine (vitamin B_2)
  - Coenzyme A – derived from panthotenic acid (vitamin B_5)

Enzyme kinetics

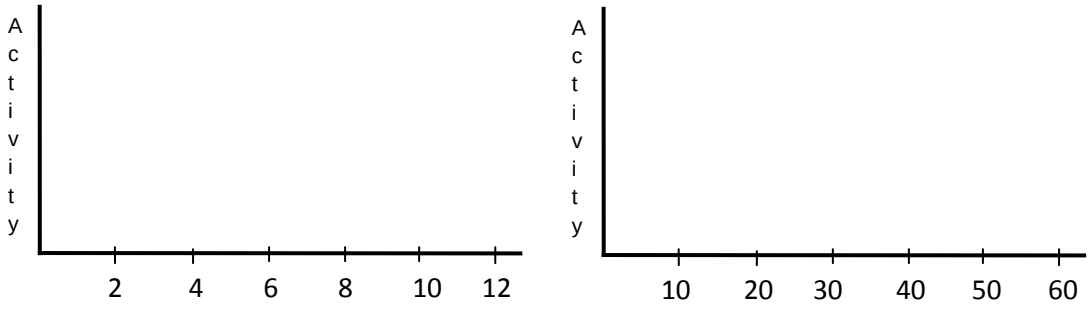
E + S ⇌ ES ⇌ E + P E = ..., S = ..., P = ...

Rate of the reaction depends on the concentration of the substrate.

At a certain concentration of a substrate the enzyme is saturated by the substrate and the rate of the reaction cannot be further increased by adding more substrate. The reaction reaches the maximum velocity.

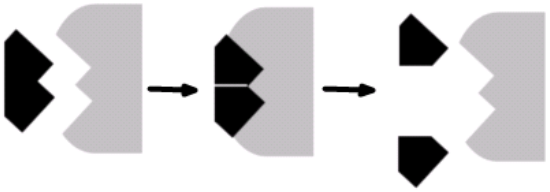


The concentration of a substrate at which the reaction goes at half of the maximum velocity is Michaelis constant (for the enzyme).It depends on the structure of the substrate, temperature and pH.



The mechanism of an enzyme function

The lock and key theory: an enzyme contains an ACTIVE SITE = BINDING SITE + CATALYTIC SITE.



2. *Suggest the type of forces which may occur between the substrate and the binding site of the enzyme.*

Induced fit theory

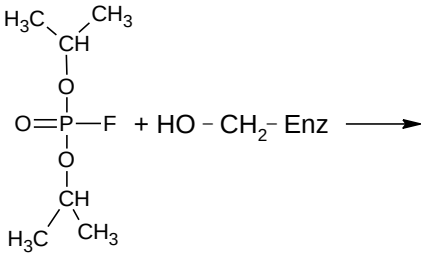
Specifity of an enzymatic reaction is based on a FLEXIBLE RESPONSE of the active site to the substrate.

http://www.teachertube.com/viewVideo.php?video_id=134992

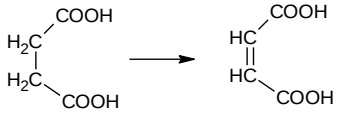
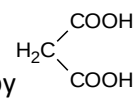
Enzyme inhibition

INHIBITOR = substance decreasing the catalytic action of an enzyme

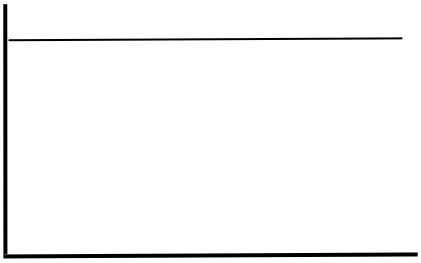
- IRREVERSIBLE: the inhibitor binds irreversibly to the enzyme by covalent bonds. The functional groups of an enzyme are changed and the enzyme loses its catalytic function.



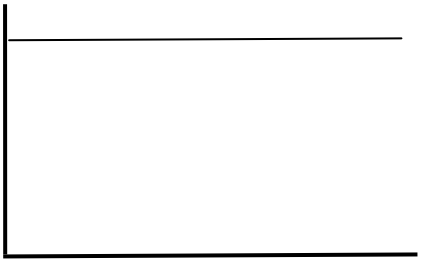
- REVERSIBLE: the inhibitor binds reversibly to the enzyme by weak forces
  - Competitive: the inhibitor combines with the active site of the enzyme and compete for it with a substrate

E.g. The enzyme for the reaction:  may be inhibited by 

- Non-competitive: in most cases the inhibitor doesn't bind to the active site of the enzyme, it binds to a different part of the enzyme



competitive inhibition



non-competitive inhibition

http://www.youtube.com/watch?v=PILzvT3spCQ&feature=related

feedback control: the final product of a sequence of enzyme catalysed reactions is an inhibitor of the enzyme catalysing the first step of this sequence. This mechanism enables to maintain certain concentrations of the produced substances.

A→B→C→D ... D is an inhibitor of E_1

http://www.youtube.com/watch?v=rHDp4wJ1U0w&NR=1

Enzymes and biotechnologies

It is expensive to isolate enzymes from cells → whole cells are used.

Proteases: hydrolyse .....

- Biological detergents
- Baking industry
- Brewing industry

Amylases: hydrolyse .....

- Baking industry
- Brewing industry
- Paper industry

Fermentation of glucose:

Using enzymes from: yeast →
bacteria →
mould →

BIOCHEMICAL PROCESSES – METABOLISM

Bioenergetics = obtaining, transfer and use of energy in living organisms

Energy is used for:

- Chemical synthesis
- Electric and osmotic work
- Mechanical work
- Heat
- Light
- Information and regulation work

Living systems do work at constant temperature and pressure ⇒ the energy changes may be described by Gibbs free energy.
$\Delta G < 0$ ... exergonic reaction – thermodynamically more probable
$\Delta G > 0$ ... . reaction – thermodynamically less probable

CATABOLIC PROCESSES: decompositions, energy is liberated, ΔG ..... 0 $kJ \cdot mol^{-1}$
ANABOLIC PROCESSES: synthetic, energy is required, ΔG ..... 0 $kJ \cdot mol^{-1}$

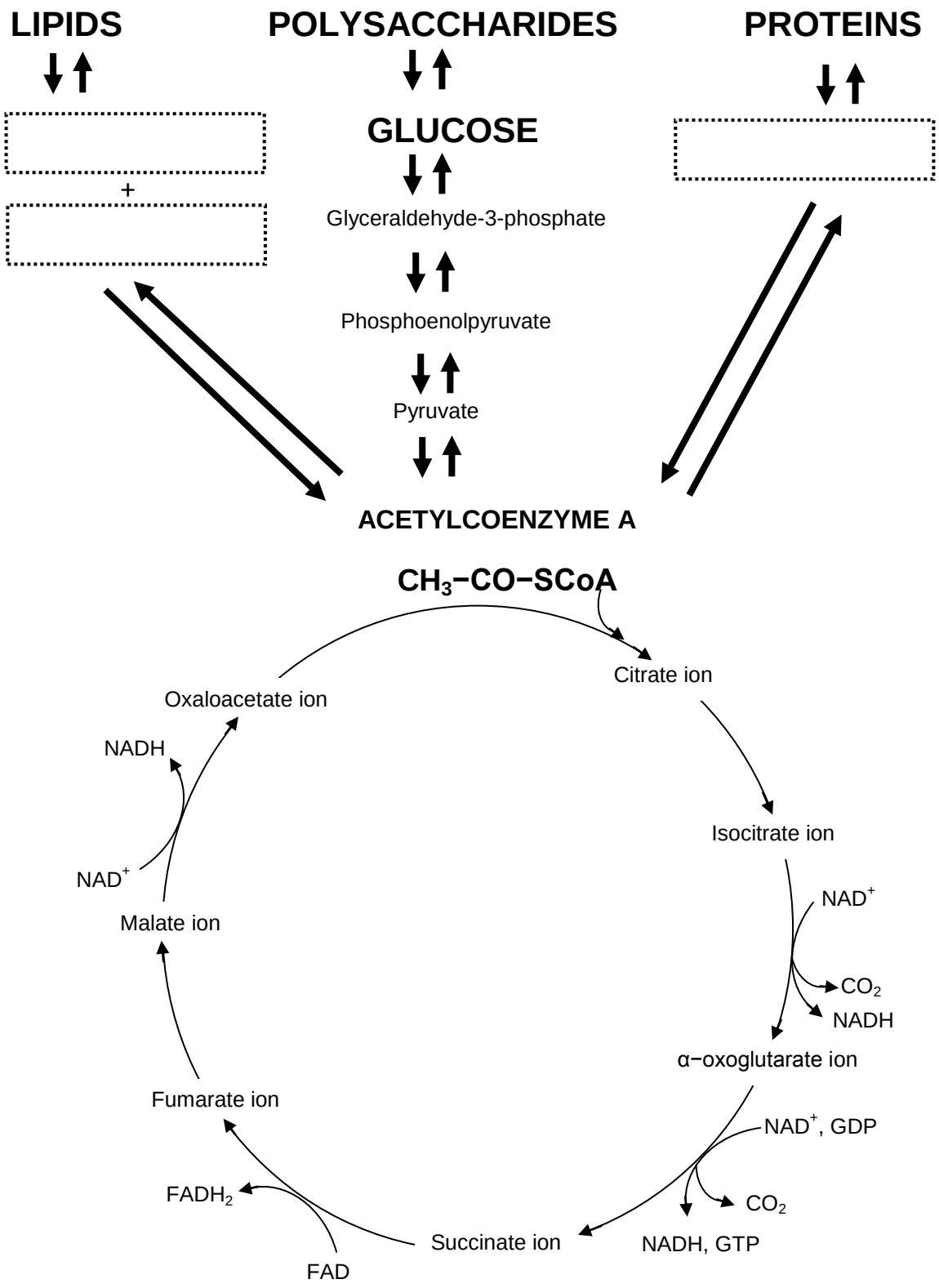
The reactions in living organisms do not occur separately, they occur in systems. Endergonic reactions take place together with exergonic. The resulting $\Delta G < 0$.

e.g. glucose + P_i ⇄ glucose-6-phosphate + H_2O $\Delta G = +13.8\ kJ \cdot mol^{-1}$
ATP + H_2O ⇄ ADP + P_i $\Delta G = -30.5\ kJ \cdot mol^{-1}$
glucose + ATP ⇄ glucose-6-phosphate + ADP $\Delta G =$ ..... $kJ \cdot mol^{-1}$

The metabolism of glucose:
$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$ $\Delta H = -2820\ kJ \cdot mol^{-1}$
In the cells it takes place as a sequence of steps, which is divided into three parts:

- **Glycolysis**: from glucose to acetyl (bonded to coenzyme A)
- **Citrate (tricarboxylic) cycle**: from acetyl to CO_2 and hydrogen bonded to NAD (FAD)
- **Electron transport chain**: hydrogen on NAD (FAD) is oxidised to water

The energy liberated in all these steps is stored in ATP (GTP): ADP + $P_i \rightarrow$ ATP $\Delta H = 32\ kJ \cdot mol^{-1}$
The energy is then liberated where it is needed: ATP $\rightarrow$ ADP + P_i $\Delta H =$..... $kJ \cdot mol^{-1}$


LIPIDS
POLYSACCHARIDES
PROTEINS
+
GLUCOSE
Glyceraldehyde-3-phosphate
Phosphoenolpyruvate
Pyruvate
ACETYLCOENZYME A
CH3−CO−SCoA
Citrate ion
Isocitrate ion
NAD+
CO2
NADH
α−oxoglutarate ion
NAD+, GDP
CO2
NADH, GTP
Succinate ion
FADH2
FAD
Fumarate ion
Malate ion
NAD+
NADH
Oxaloacetate ion

The electron transport chain (respiratory chain)

-there are four dehydrogenation steps in each citrate cycle:

3 times $NAD^+ + 2H \rightarrow NADH + H^+$

$FAD + 2H \rightarrow FADH_2$

The hydrogen atoms are oxidised by molecular oxygen to form water. The oxidation takes place by means of a series of electron transfers.

$NADH + O_2 \rightarrow$

$FADH_2 + O_2 \rightarrow$

Energy is liberated and stored in .....

Most of the ATP formed through the oxidation of glucose is produced in the electron transport chain.

3. *Make the formulae for the ions taking part in the citric cycle:*

phosphoenolpyruvate *citrate* *isocitrate*

α-glutarate *succinate* *fumarate*

malate *oxaloacetate*

knowing that: pyruvic acid = 2-oxopropanoic acid

α -glutaric acid = 2-oxopentanedioic acid *citric acid = 2-hydroxypropane-1,2,3-tricarboxylic acid*

fumaric acid = trans butanedioic acid *succinic acid = butanedioic acid*

oxaloacetic acid = oxobutanedioic acid *malic acid = hydroxybutanedioic acid*

4. *Classify the enzymes catalysing the steps of the citric cycle:*

1 *4*

2 *5*

6