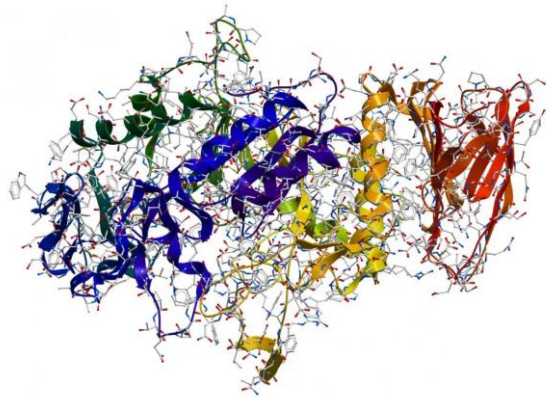


CEN-CHE 422
ENZYME ENGINEERING

ENZYME STRUCTURE AND CLASSIFICATION

Molecular Structure of Enzymes

Enzymes are high molecular weight proteins that have catalytic activity
($15{,}000 < MW < 10^6$ Daltons)

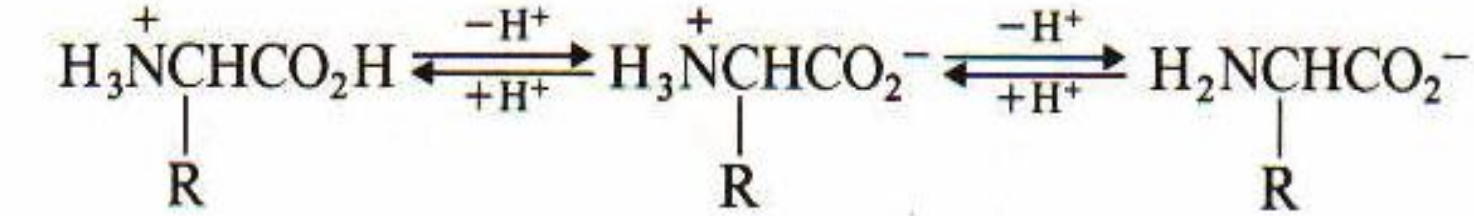
- **Proteins are made up of amino acids linked by peptide bonds.**
- **Different proteins are formed by the binding of different amino acids in different order.**
- **Amino acids are the basic building blocks of proteins.**
- **There are twenty naturally occuring amino acids.**
- **Amino acids are chiral (optically active, L- and D-isomers) compounds (other than glycine) that have carboxyl and amine groups in their structure.**
- **The R group attached to the assymetric carbon atom is different in all amino acids.**

AMPHOTERIC NATURE OF AMINO ACIDS

Amino acids are amphoteric, which means they have acidic and basic tendencies.

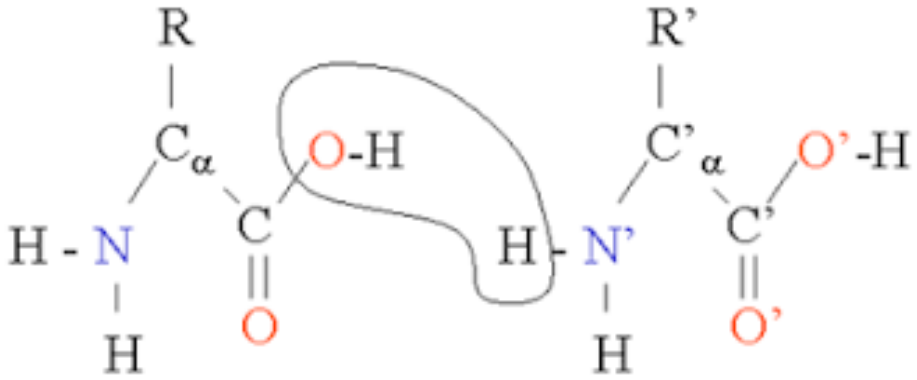
The carboxyl group is able to lose a proton and the amine group is able to accept a proton.

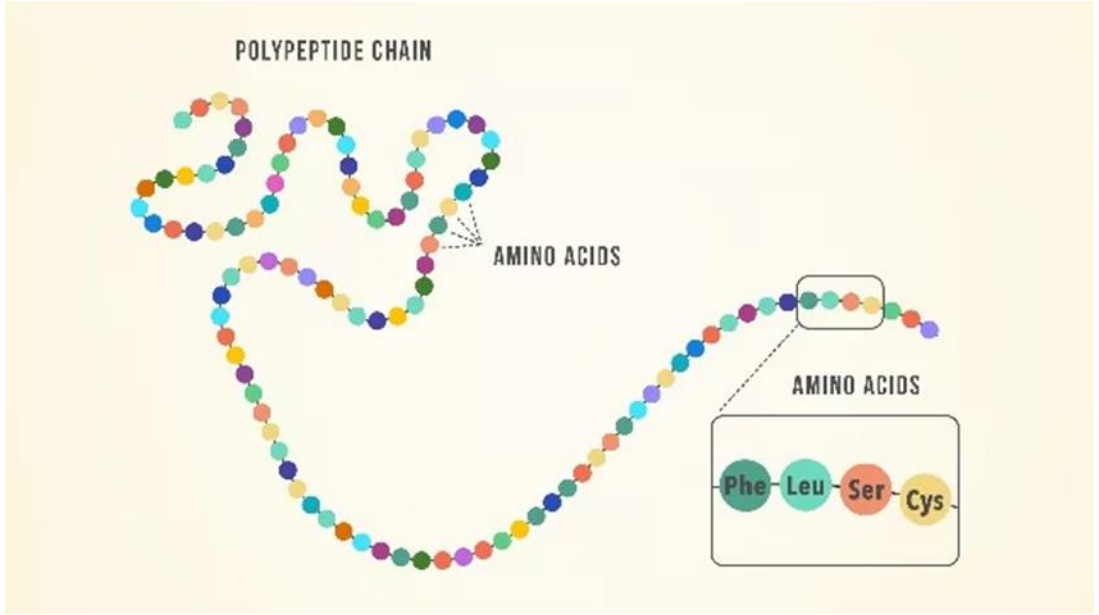
Amino acids are also ionic in character, and behave as ampholytes, meaning they move to their isoelectric points when placed in a pH gradient under an electric field.

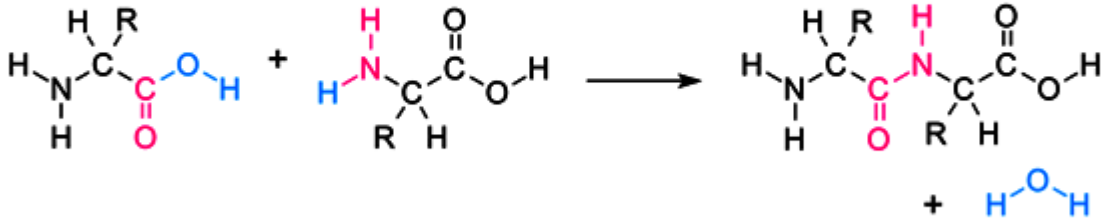


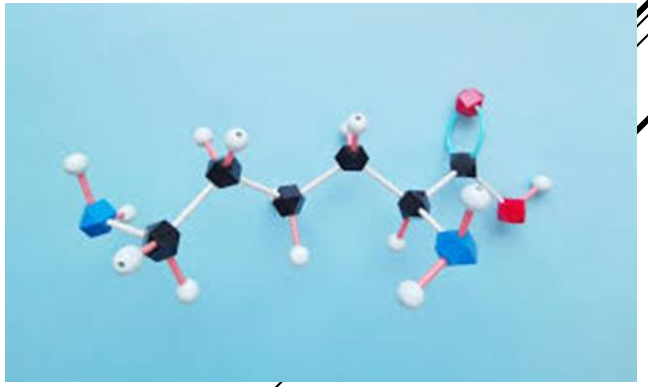
Katyon formu	Dipolar iyon	Anyon formu
pH<pI	pH=pI	pH>pI

Proteins


R
Cα
O-H
H - N
C
O
H
R'
C'α
O'-H
H - N'
C'
O'
H


POLYPEPTIDE CHAIN
AMINO ACIDS
AMINO ACIDS
Phe
Leu
Ser
Cys





Protein structures

Primary structure: The linear sequence of amino acids.

Secondary structure: Regular, recurring arrangements in space of adjacent amino acid residues in a polypeptide chain. It is maintained by hydrogen bonds between amide hydrogens and carbonyl oxygens of the peptide backbone. The major secondary structures are α-helices and β-structures.

Tertiary structure: The three dimensional shape of a protein

Quaternary structure: The association of several protein chains or sub-units into a closely packed arrangement

Cofactor

95% of enzymes is protein. This protein has no catalytic activity. Catalytic activity of the enzyme is supplied by cofactor. Without cofactor, enzymes cannot show any catalytic activity. Almost all enzymes have cofactor. But there area some exceptions such as lipase.

A cofactor is a non-protein chemical compound or metallic ion that is required for an enzyme's role as a catalyst

All proteins are not enzyme.

1. Metal ions:

Cofactor	Enzyme
Ca^{+2}	α-amylase Collagenase
Co^{+2}	Glucose isomerase
Cu^{+2} (Cu^{+})	Galactose oxidase Triosinase
Fe^{+2} veya Fe^{+3}	Catalase Peroxidase
Mg^{+2}	Deoxycarboxcylase
Mn^{+2}	Arginase
Na^{+}	Plasm membrane ATPase (with K^{+} and Mg^{+2})
Zn^{+2}	Alcohol dehydrogenase Alkaline phosphatese Carboxypeptidase

2. Coenzymes:

Complex organic molecules

Vitamins and vitamin derivatives

NAD , NADP ⇒ alcohol dehydrogenase

FMN, FAD ⇒ glucose oxidase

CoA pyridoxal 5 phosphate, biotin

3. Prostetik groups:

Compounds tightly bound to enzymes

Dihidroalanin, Vitamin B6, Vitamin C

Naming of Enzymes

Enzymes are naming by adding suffix -ase to

→ substrate converted (e,g, urease)

→ reaction catalyzed (e,g, dehydrogenase)

urease : decomposition of urea into ammonia and CO_2

alcohol dehydrogenase : dehydrogenation of alcohol

lactase : decomposes lactose into glucose and galactose

fumarase : converts fumarate to malate

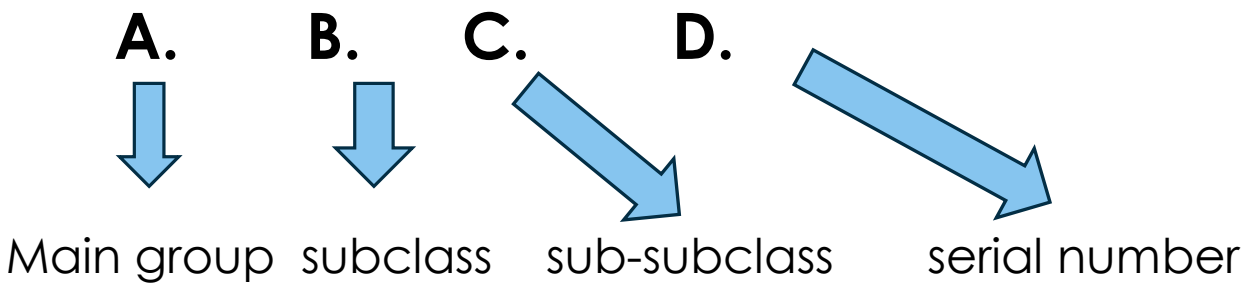
Some enzymes have arbitrary names (proteolytic enzymes: pepsin, trypsin, rennin).

Classification of Enzymes ve Codes

Enzyme are specific molecules and classified according to the reactions they catalyse.

Enzyme Commision (E.C.) classified enzymes into 6 groups.

Every enzyme has a specific code. It consists of the letters "EC" followed by four numbers separated by periods.
EC 1.2.1.5



Class	Group	Function	Sub-group
Oxidoreductases	1	Oxidation and reduction reactions	1. 1. acting on CH-OH 1.2.acting on C=O 1.3.acting on C=C
Transferases	2	Transfer of functional groups X-Y + Z → X + Y-Z	2.1. one-carbon groups 2.2 Aldehydic or kentonic groups
Hydrolyses	3	Hydrolysis reactions	3.1.Esters 3.2. Glycosidic bonds

Sınıf	Grup	Fonksiyon	Altgrup
Liyases	4	Addition to double bands	4.1.C=C 4.2.C=O
İsomerases	5	Isomerisation reactions	5.1 racemases 5.2. cis-trans
Ligases	6	Formation of bonds with ATP cleavage	6.1.C-O 6.2 C-S

Intracellular and Extracellular Enzymes

Enzymes are also classified as introcellular and extracellular enzymes

Extracellular enzymes are secreted out of the cells; it is easier to prufy extracellular enyzmes

Catalytic activity of enzymes

$$E + S \Leftrightarrow ES \rightarrow E + P$$

The shapes of the enzyme and substrate match each other exactly

The substrate is the chemical that the enzyme acts on (reactant)

Enzymes are specific catalysts (only one enzyme acts on only one substrate)

The active site of the enzyme is the place where the reaction occurs

On the active sides:

The reactive groups of enzymes:

R (residual) groups of Asp, Glu, Cys, His, Lys, Met, Ser, Trn; end amine ve carboxyl groups. **Among a dozen amino acid residues only 2 or 3 take place in substrate binding.**

Active sites occupies only about 5% of the total surface area of the enzyme

Active sites have specific structures

A change in the shape of protein affects the shape of active site and therefore the function of the enzyme

Fisher’s key and lock model:

In this model, the active site of the enzyme does not form a pit; however, upon contact with the substrate, the amino acid R- groups are arranged to bind the substrate.

Koshland’s induced-fit model

This model assumes that the active site in the enzyme is similar in size, structure, and chemistry to the substrate molecule.

Specificity of Enzymes

Absolute specificity - the enzyme will catalyze only one reaction (urease)

Group specificity - the enzyme will act only on molecules that have specific functional groups (such as amino, phosphate and methyl groups)

Linkage specificity - the enzyme will act on a particular type of chemical bond regardless of the rest of the molecular structure. (peptidase acts on peptide bonds; phosphatase acts on phosphate esters; esterase acts on carboxylic esters

Stereochemical specificity - the enzyme will act on a particular steric or optical isomer (L-; D-; cis; trans)

Benefits over Chemical Catalysis

Enzymes and **catalysts** both affect the rate of a reaction. In fact, all known enzymes are catalysts.

Enzymes are largely organic in nature and are bio-catalysts, while non-enzymatic catalysts can be inorganic compounds.

Neither catalysts nor enzymes are consumed in the reactions they catalyze.

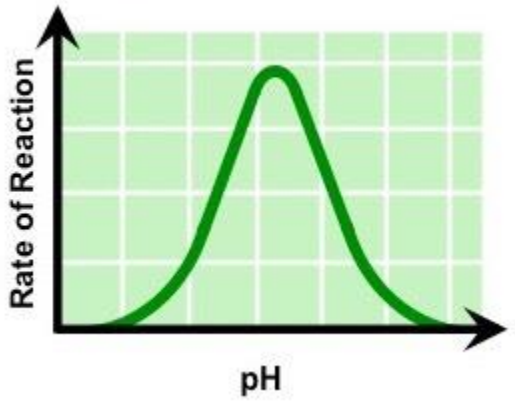
Enzyme Activity

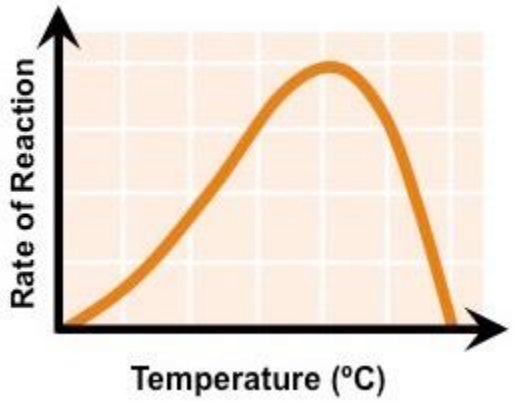
Enzyme Unit (U) is defined as the amount of the enzyme that catalyzes the conversion of one micromole of substrate (or production of one micromole of product) per minute under the specified conditions (pH, T) of the assay method.

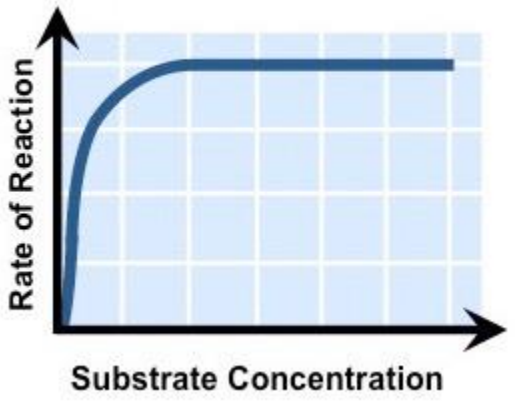
Example: One unit of glucoamylase enzyme activity is defined as the amount of enzyme required to produce one micromole of glucose per min in a 4% solution of Lintner starch at pH 4.5 and 60°C.

Specific enzyme activity (simply as 'specific activity') (U/mg protein) is the number of enzyme units per ml divided by the concentration of protein in mg/ml

Factors Affecting Enzyme Activity







4) Enzyme Concentration: Increasing the enzyme concentration at a constant substrate concentration increases the enzymatic reaction rate.

5) Salt concentration: Each enzyme has an optimum salt concentration.

6) Inhibitors: Inhibitors in the environment reduce its activity by acting on the enzyme through various mechanisms.