Physico-chemical properties of peptides and proteins

The aim:

- the examination of physico-chemical properties of proteins based on processes of salting and precipitation
- the determination of isoelectric point of selected protein

Peptides and proteins

- Peptides contain from 2 to 100 aminoacids bound by peptide bonds. They can be divided into oligopeptides (2-10 aminoacids) and polypeptides (11-100 aminoacids)
- Proteins contain more than 100 aminoacids in molecule but differ from peptides not only with the size of molecule but also physical properties
- Proteins in contrary to peptides do not cross semipermeable membranes but undergo denaturation

Peptides: division and meaning

- Oligopeptides (2-10 aminoacids in chain):
  - carnosine and anserine (contain β-alanine)
  - glutathione (tripeptide: γ-glutamyl-cysteinyl-glycine) – participates in redox reactions
  - vasopressin and oxytocin (ring nanopeptides) – hormones of pituitary gland

- Polypeptides (11-100 aminoacids in molecule):
  - insulin (2 chains; 51 aminoacids) pancreatic hormone
  - glukagon (29 aminoacids) pancreatic hormone
  - other hormones

Criteria for protein division

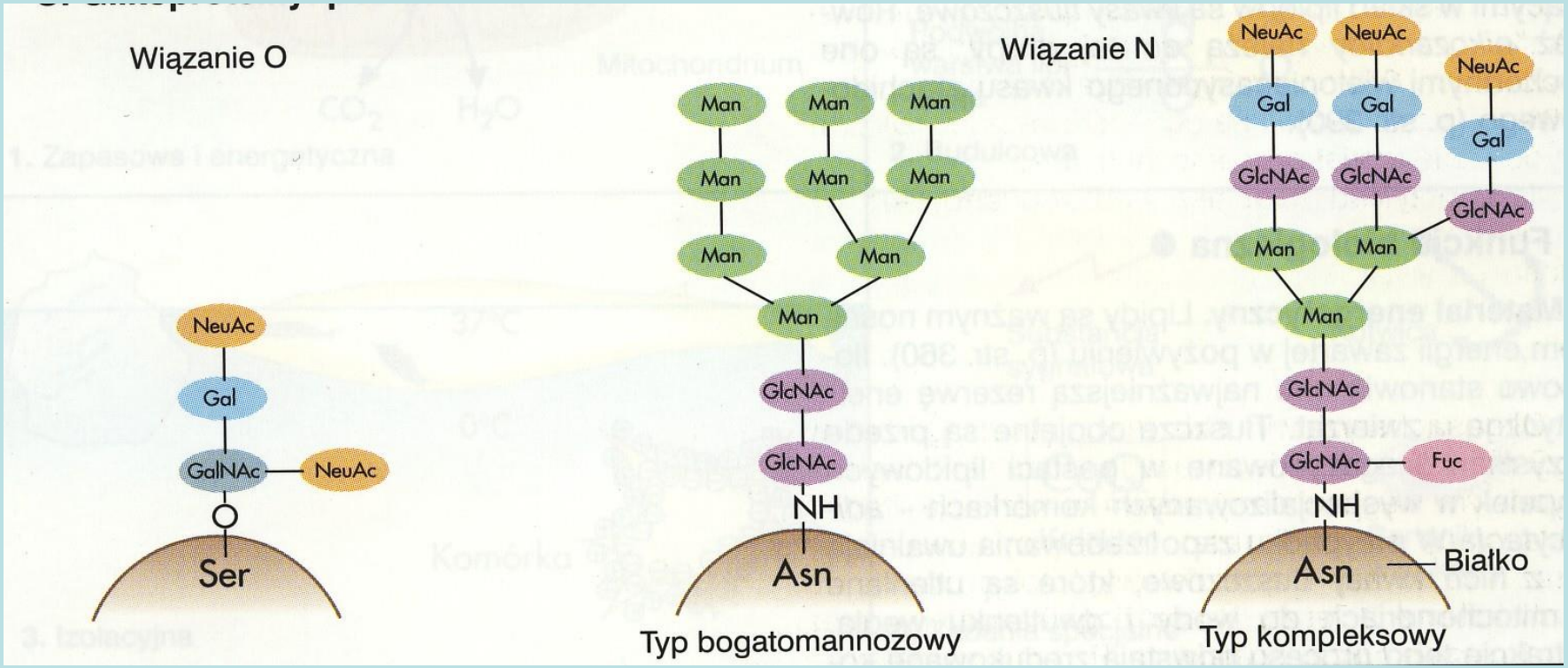
- origin: animal and plan proteins
- occurence: proteins of blood plasma, muscles, milk, cell..
- Biological functions:

  - regulatory proteins: enzymes, hormones
  - structural proteins: connective tissue, fibrinogen
  - transport proteins: Hb, plasma albumins, components of oxidative chain which transport electrons
  - storage proteins: ferritin, ceruloplasmin
  - contractile proteins: actin i myosin
  - receptor proteins

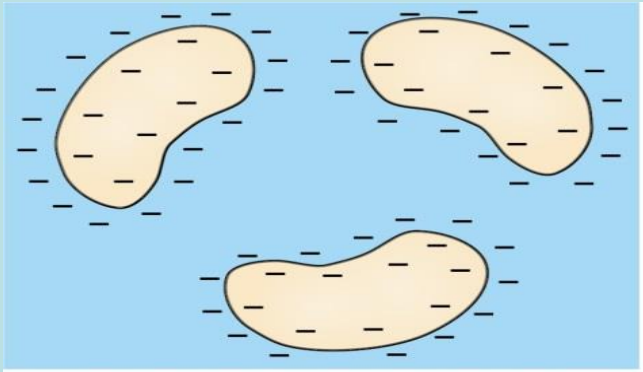
Criteria for protein division

- In accordance to chemical composition:
  - single proteins (contain only aminoacids)
  - conjugated proteins – contain additional compounds:
    - phosphoproteins
    - glycoproteins
    - chromoproteins (np. Hb)
    - jonoproteins
- In accordance to the shape and solubility of molecule:
  - globular proteins
  - fibrous proteins

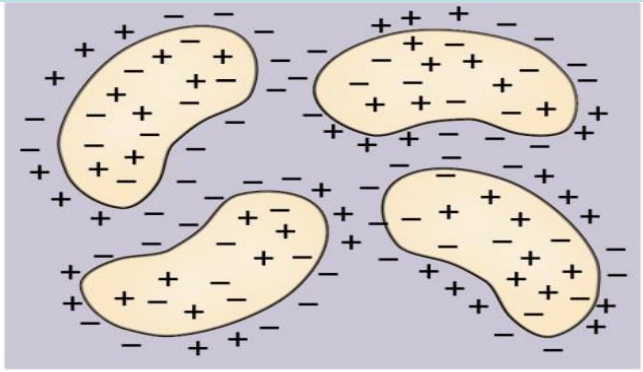
Example: glycoproteins



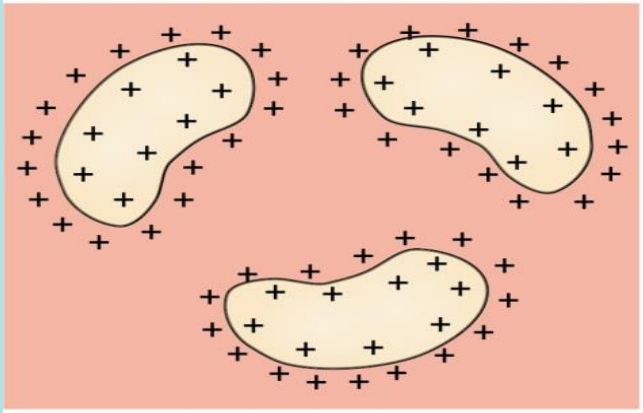
Salting in-out of protein



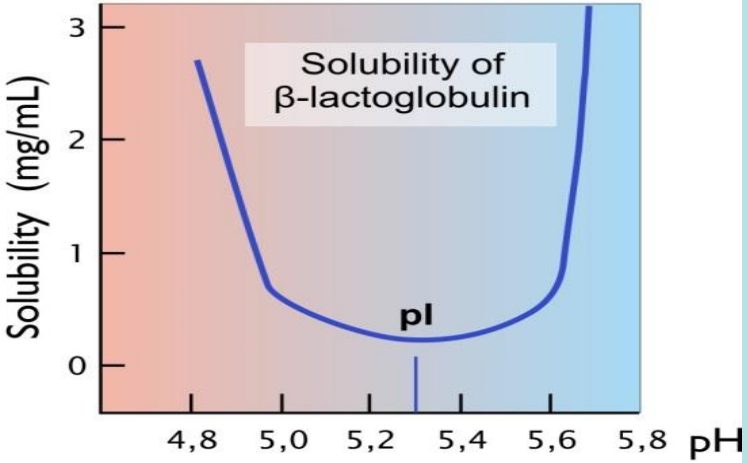
a) At pH values above the isoelectric point the protein is negatively charged



b) pH=pI, the number of negative and positive charges is equal



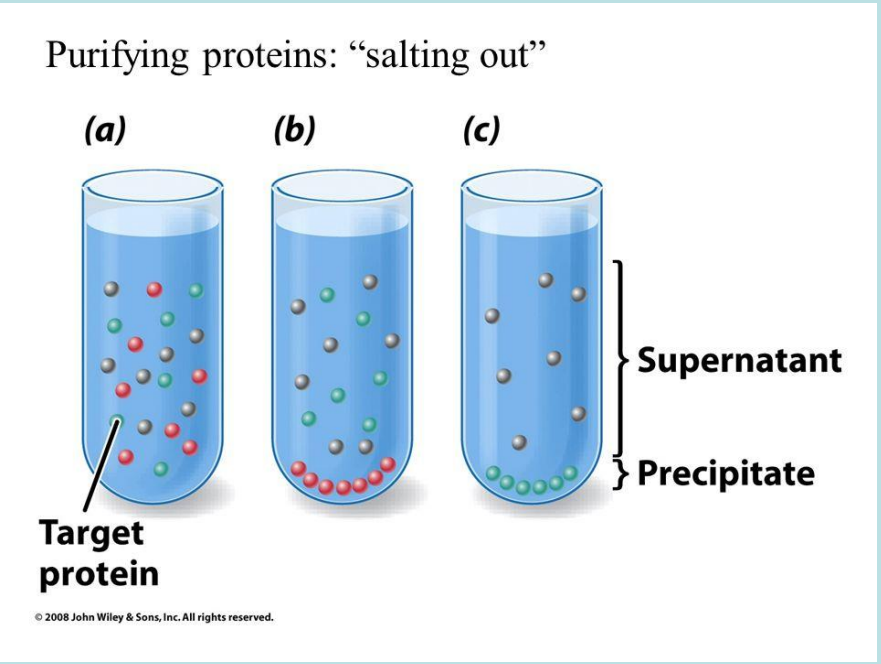
c) At pH values below the isoelectric point the protein is positively charged

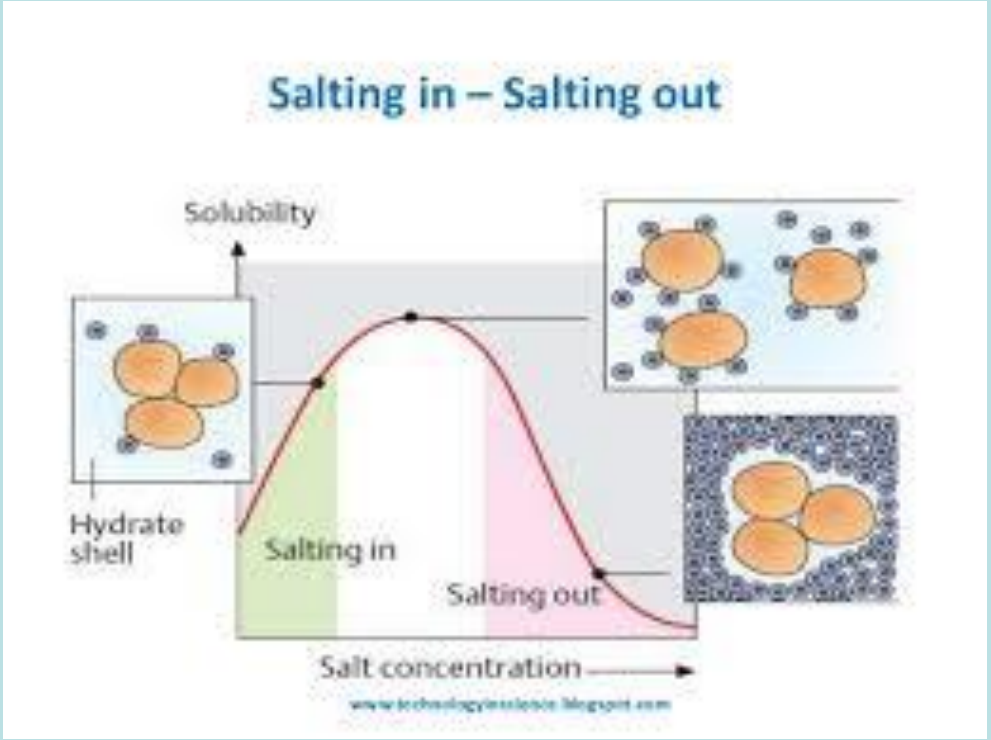


d) pH-dependence of the solubility of the β-lactoglobulin protein

The increase of solubility (salting in) of proteins

Some of proteins can be poorly soluble in water. But the addition of small amounts of salts to water solution of proteins may increase the solubility. Optimal concentration of salt which may assure maximal solubility of plasma proteins is 0,9% NaCl. This solution of sodium chloride is called physiological solution (physiological salt).





Salting out of proteins from the solution

- The process of precipitation of protein from the solution by use of increasing concentrations of inorganic salts (eg. ammonium sulphate) acetone, cold alcohol.
- Salting out is related to the removal of water shell surrounding the molecule of protein – molecules without this shell aggregate due to their own charges and drop to the bottom of tube.
- It is reversible process – the addition of water can reverse it. It does not harm protein molecule, does not result in the loss of biological activity because does not damage protein structure.
- It may be useful in the separation of different proteins in the solution
- It is the easiest to salt out proteins in their isoelectric point

Isoelectric point

- This term concerns polyfunctional compounds with amphoteric character (aminoacids, peptides, proteins). These groups can dissociate in water environment and obtain particular charge. It depends on pH of solution. For each of these molecules particular **pH** when the sum of positive and negative charges is balanced (equals zero) and the molecule possesses neutral charge can be designated.
- In isoelectric point the molecule does not move in electric field, is hardly soluble and easily precipitates from the solution
- In isolelectric point molecules are not surrounded by water shell, do not repel each other and easily form aggregates

Denaturation of protein

- Denaturation consists in the damage to spatial structure of protein but primary structure is preserved
- Denaturated protein looses its biological properties and is not able to function any more. Soluble proteins may lose their solubility
- Denaturation factors:

  - physical: elevated temperature, UV radiation, mechanical factors
  - chemical: organic solvents, acids, bases, heavy metal ions, concentrated solutions of urea or guanidine hydrochloride

Task 1. The determination of the isoelectric point of protein

Protocol:

To 6 tubes, measure the volumes of standardized solutions of CH3COOH and distilled water given in the table, thus creating different reaction environments. Then add 1cm3 of 0,5% solution of Casein in 0,1mol/dm3 CH3COONa to each tube. After adding each drop, shake the tube. Set aside the tubes for 30 minutes and after that time observe changes in the turbidity of the solutions. Make note of the results in the table: no turbidity (-) or different degrees of turbidity (+,++,+++). Calculate the pH in each tube using the Henderson-Hasselbalch equation.

In which pH is the highest turbidity? What is the value of isoelectric point for Casein?

Składnik, cm^3	Nr probówki					
	1	2	3	4	5	6
0,01M CH_3COOH	0,6	1,3	–	–	–	–
0,1M CH_3COOH	–	–	0,3	1	8	–
1M CH_3COOH	–	–	–	–	–	1,6
H_2O	8,4	7,7	8,7	8	1	7,4
0,5% kazeina	1	1	1	1	1	1
Zmętnienie						
pH						

Task 2. Salting out of Albumins and Globulins

The aim of the test is to separate the mixture of serum proteins through the salting out process, which uses the chemical properties of individual proteins.

Protocol: Add an equal volume of saturated solution of (NH4)2SO4 to 5cm3 of serum. Filter the solution after precipitation of the flocculent globulin sediment. Add (NH4)2SO4 *in substantia* in portions to the filtered supernatant and shake the tube until at the bottom of tube crystals of insoluble (NH4)2SO4 will remain. The sediment of albumins is precipitated. After filtration, examine the solubility of the sediment in H2O. Check the filtrate for protein presence using precipitation method with trichloroacetic (TCA) or sulfosalicylic acid.

Task 3. Precipitation of proteins using inorganic and organic acids

The aim of the test is to observe the action of concentrated inorganic and some organic acids on proteins. These reactions may result in protein denaturation.

Protocol: For each of 3 test tubes add 1cm3 of 1% egg white solution. Then, to 1,2 and 3 tube enter the 0,5cm3 of concentrated HCl, HNO_3 and H_2SO_4 respectively. Shake the contents of each tube. In test tubes containing HCl and H_2SO_4, the sediment of denatured protein dissolves (salts of proteins with these acids strongly dissociate). However, in test tube containing HNO_3, the sediment doesn't disappear after shaking.

Add 2cm3 of 1% egg white solution to two other tubes. Then add dropwise 5% TCA solution to the 1 tube and 20% sulfosalicylic acid solution to 2 tube. A white sediment is precipitated. TCA and sulfosalicylic acid form insoluble salts with proteins and therefore they are used to remove proteins from biological fluids. These compounds in higher concentrations cause protein denaturation.

Task 4. Precipitation of proteins using heavy metal cations

The aim of the test is to observe the action of heavy metal salts (Cu, Hg, Pb) on proteins depending on pH.

Protocol: To 3 test tubes add 2cm3 of 1% protein solution at pH=3 and to other 3 tubes add 2cm3 of the same proteins at pH=8. Then to each tube add dropwise (avoid excess of the reagent!) reagents listed in the table below. Record in the table the formation (+) or absence (-) of sediment. Explain the reaction results in each tube, taking into account the environment and ionizing of the relevant groups.

REAGENT	EGG WHITE	
	pH=3	pH=8
10% $CuSO_4$		
5% $HgCl_2$		
5% $(CH_3COO)_2Pb$		

Denaturation of protein