Series No. 4

Structures and Physicochemical Properties of Amino Acids, Peptides and Proteins

Exercise 01 :

Using the given pKa values, calculate the pHi (isoelectric point) of the following amino acids:

	pKa	pKb	pKr
Ala	2.34	9.69	/
Thr	2.63	10.43	/
Asp	2.09	9.82	3.86
Lys	2.18	8.95	10.53

Exercise 02:

Given the following amino acids: Ala, Asp, Lys.

- Write the ionization equation of these amino acids.
- Calculate their pI (isoelectric point)?
- Indicate the overall net charge of these amino acids at pH=1, pH=6, and pH=10.
- Do they possess optical rotation (optical activity)? Justify.

Exercise 03 :

1. What is the electrical charge (+, 0, -) of the following amino acids: Glycine, Serine, Aspartic acid, and Arginine at : : (a) pH 2,00 ; (b) pH 3,96, (c) pH 5,68 ; (d) pH 10,76.

	Glycine pHi=5,97	Serine pHi=5,68	Aspartic acid pHi=2,97	Arginine pHi=10,76
pH 2,00				
pH 3,96				
pH 5,68				
pH 10,76				

2. A mixture of these amino acids is separated by anion exchange chromatography. What will be the elution order of the amino acids if we use a buffer gradually changing from pH 12 to pH 4?

Exercise 04 :

Consider a mixture of three peptides:

A = Lys-Ala-Arg B = Glu-Leu-Asp C = Ala-Gly-Ser

1. Calculate their pI (isoelectric point).

The mixture is subjected to electrophoresis at **pH=6**

1. Indicate on a diagram the position of the three peptides at the end of the experiment. Justify the result.

The same mixture is loaded at the top of a column filled with polystyrene resin substituted with sulfonate groups **(SO3-)**, applying a pH gradient from **2** to **13**.

1. What type of ion exchange chromatography is used?
2. Indicate the elution order of the three peptides.
3. Can these peptides be separated when loaded on the same column, but this time applying a pH gradient from **13** to **2**?

Exercise 05

After partial hydrolysis by trypsin of a polypeptide, a tetrapeptide composed of amino acids Val, Arg, Cys, Glu was isolated.

The N-terminal amino acid plays an important role in the formation of inter-chain and intra-chain covalent bonds in proteins.

The following amino acid migrates closest to the anode at pH=8.5 compared to the other amino acids in the peptide.

1. Give the sequence of the tetrapeptide.
2. Write its chemical structure and give its nomenclature.
3. How does its ionization state vary when the pH changes from an acidic to a basic medium? Calculate its pHI.

Given pK values:α-NH2=10.7 ; α-COOH=1.8 ; Thiol= 8.33 ; γ-COOH= 4.1 ; Guanidinium=12.4.

Exercise 06

A polypeptide P composed of **12 amino acids** contains among others **lysine** and **aspartate** as the only charged polar amino acids. The AA composition of P after acid hydrolysis shows **no aromatic amino acids**.

1- The action of trypsin on P gives in order (from N-terminal end) the following fragments: T1 (**Tripeptide**); T2 (**Tripeptide**); T3 (**Hexapeptide**).

2- The digestion of P by chymotrypsin also gives in order: CT1 (**Tetrapeptide**); CT2 (**Pentapeptide**) and CT3 (**Tripeptide**).

3- Treatment with CNBr of P gives in order: CN1 (**Pentapeptide**); CN2 (**Tripeptide**) and CN3 (**Tetrapeptide**).

4- The action of **Sa protease** on P gives in order: SP1 (**heptapeptide**), SP2 (**Tripeptide**) and two free amino acids.

5- Edman degradation on P gives **an amino acid then PTA-Ala**.

6- The action of Dansyl chloride on P gives **dansyl-Gly**.

7- Its treatment with carboxypeptidase gives a **non-chiral amino acid**.

8- The following information is provided: T1 and CT1 have the same N-terminal end. T3 and CT3 have the same N-terminal end.

Question: Deduce the sequence of P.

Exercise 06

A hexapeptide is subjected to total acid hydrolysis. The acid hydrolysate contains: Arg, Ala, Val, Leu, Phe, Tyr.

1. The aminopeptidase method shows that the NH2-terminal amino acid is Ala.

2. Tryptic hydrolysis leads to two tripeptides, one of which consists of Ala, Arg, and Tyr.

3. Partial acid hydrolysis leads to a mixture of peptides:

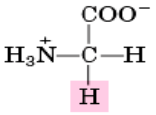
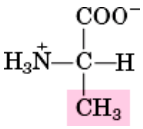
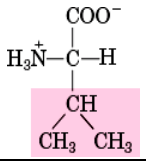
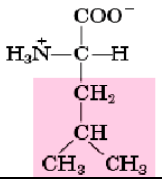
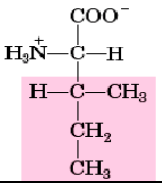
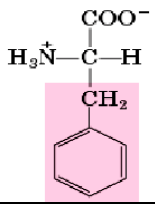
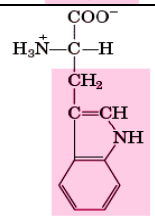
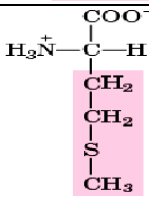
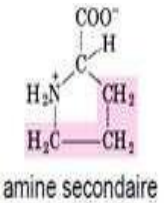
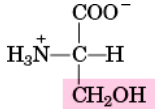
Peptide P1 consisting of Arg and Phe

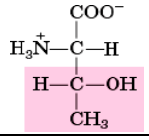
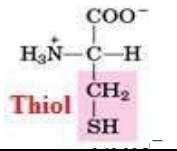
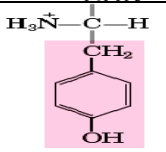
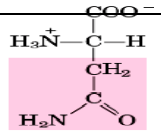
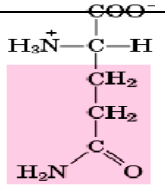
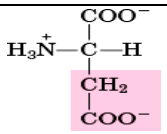
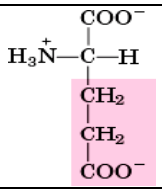
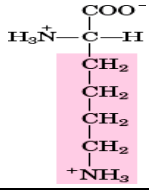
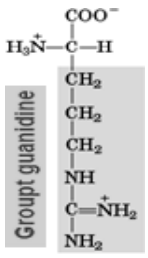
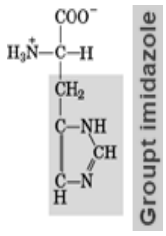
Peptide P2 consisting of Leu and Val

Peptide P3 consisting of Phe, Leu, and Arg

- Deduce the amino acid sequence of the hexapeptide.

Table I: Classification of the 20 AA according to polarity and group capacity to take an electric charge

Polarity	Electric Charge	Name	Symbol	Structure	pk_1	pk_2	pk_3	pH_i
AA Non polar = Hydrophobic	Not Charged	Glycine/ Glycocolle	Gly [G]		2.4	9.8	/	6.0
		Alanine	Ala [A]		2.4	9.9	/	6.0
		Valine	Val [V]		2.2	9.7	/	6.0
		Leucine	Leu [L]		2.3	9.7	/	6.0
		Isoleucine	Ile [I]		2.3	9.8	/	6.1
		Phénylalanine	Phe [F]		2.2	9.2	/	5.5
		Tryptophane	Trp /Try [W]		2.4	9.4	/	5.9
AA Non polar = Hydrophobic		Méthionine	Met [M]		2.1	9.3	/	5.8
		Proline	Pro [P]		2.0	10.6	/	6.3
		Serine	Ser [S]		2.2	9.2	/	5.7

AA Polar = Hydrophilic	Neutrals (Not Charged)	Thréonine	Thr [T]		2.1	9.1	/	6.5
		Cystéine	Cys [C]		1.9	10.8	8.3	5.0
		Tyrosine	Tyr [Y]		2.2	9.1	10.1	7.4
		Asparagine	Asn [N]		2.1	8.8	/	5.4
		Glutamine	Gln [G]		2.2	9.1	/	5.7
	Acids Negatively Charged	Acide Aspartique	Asp [D]		2.1	9.9	3.9	3.0
		Acide Glutamique	Glu [E]		2.1	9.5	4.1	3.2
	Bases Positively Charged	Lysine	Lys [K]		2.2	9.2	10.8	9.8
		Arginine	Arg [R]		1.8	9.0	12.5	10.8
		Histidine	His [H]		1.8	9.3	6.0	7.6