University of Batna2
Faculty of natural and life sciences
Tutorials of Biochemistry
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Series of Biochemistry tutorials CHAPTER1: Proteins and aminoacids

Exercise 01

The pKs of the α-COOH and α-NH2 groups of L-Alanine are respectively pK1= 2.34 and pK2=9.69. Demonstrate the pHi value?

Exercise 02

A mixture of Glu, Leu, Lys whose pHi are respectively 3.22; 5.98; 9.74 is subjected to electrophoresis at pH6. Give the developed structure of these AA, indicate towards which poles these AA migrate, justify the answer?

Exercise 03

We want to separate the Glu, Leu and Lys by chromatography on polystyrene resin substituted by sulfonate groups (-SO3-). The pHi of Glu, Leu and Lys are respectively: 3.22; 5.98; 9.74 at 25°C. These aa are placed on the column at pH2 and then the pH is gradually brought to 7. Which aa are eluted and in what order.

Exercise 04

Consider the following dipeptides: The dipeptide MM Phi His-Lys 283 9.85 Pro-Arg 271 11.54 Glu-Glu 204 3.22 Calculate their electrophoretic mobility at pH 9.85 Indicate the direction of migration of the dipeptides Answer: Mobility = pH- pHi/MM; the sign of the mobility is the sign of the pole towards which the dipeptide migrates.

Exercise 05

We are given the following peptides: Lys-Ala-His-Gly-Met and Trp-Leu-Asp-Cys. Write the structural formula of these peptides. Study the variation of their net charge as a function of pI (pH) and determine their isoelectric pI. The following values will be used, for pKa of the different ionizable functions:

Function	α- COOH	β or γ-COOH	α-NH2	ε-NH2	Imidazole	SH
pKa	3	4.5	8.5	10.5	6.5	10

Exercise 06

Draw the structure of the peptide GWYQR. Indicate the ionized form of this peptide at the following pHs: a) pH 2; b) pH 7: c) pH 10.5

Exercise 07

After hydrolysis by chymotrypsin of the protein “0.62” from wool, an oligopeptide P was obtained whose amino acid composition is: Thr 1, Ser2, Pro1, Gly1, Val1, Cys1, Phe1, Tyr1. The Edman reagent allows us to successively obtain from P, the PTHs of Ser, Ser, The, and Val. Thermolysin, a proteolytic enzyme, has notably made it possible to obtain, from P, a shorter oligopeptide, P’, from which the PTHs of Phe, Pro, Gly and Cys are successively detached with the Edman reagent. What is the sequence of P.

Exercise 08:

After tryptic hydrolysis of the L7 protein of the large ribosomal subunit of E. coli, an oligopeptide P was isolated, whose amino acid composition is: Lys1, Asx1, Thr1, Glx1, Val1, Leu1, Ile 1, phe1. The net charge of P is (-) at pH 6.5. After the action of dansyl chloride on P, then acid hydrolysis, dansylthreonine is identified. Carboxypeptidase successively detaches from P: Lys, Leu, Ile, and Val. When P is hydrolyzed by chymotrypsin, an oligopeptide P' is obtained, whose amino acid composition is: Asx1, Val1, Leu1, Ile1. What is the sequence of P?

Exercise 09

Myoglobin contains 0.335% iron (Mr = 56 g / mole). Calculate its minimum molecular weight. The data 0.33% iron means: 0.33 g iron in 100 g myoglobin. Moreover: 56 g iron contains 1 mole of myoglobin.

Exercise 10

We determine the elution volume, Ve during a Sephadex chromatography of the following proteins whose MW is known.

Enzymes	Mr	**Log Mr**	Volume d'élution (Ve), ml
Aldolase	145000	5.16	52
Lactate déshydrogénase	135000	5.13	57
Phosphatase alcaline	80000	4.9	92
Ovalbumine	45000	4.65	131
Lactoglobuline	37100	4.57	143

- Plot (draw) Ve versus log Mr, what do you notice?
- If glucokinase is among these proteins mixture, that it we be elueted at **Ve= 105** ml, determine its molecular weight, using the obtained standard graphic?

Exercise 12

A DEAE-cellulose column at pH 6.5 is loaded with a mixture of proteins: ovalbumin (pHi 4.6), urease (pHi 5.0), and myoglobin (pHi 7.0). The column is eluted with a low ionic strength buffer at pH 6.5, then with the same buffer containing increasing concentrations of sodium chloride. In what order will the proteins appear in the eluent?

DEAE-cellulose