Graduate Program In Biochemistry (IQ-USP) - Admission Test
July-2021

Applied online

Q1 A sample of **peptide A**, whose structure is shown in the scheme below, was treated with pepsin. Another sample of the same peptide was trypsinized. After treatment, enzymes were discarded. The material resulting from each treatment was subjected to electrophoresis at pH 7.4. A sample of **peptide A** was also subjected to electrophoresis at pH 7.4. In the peptide sequence shown below, the starting NH2 and the terminal COOH are shown in the peptide structure.

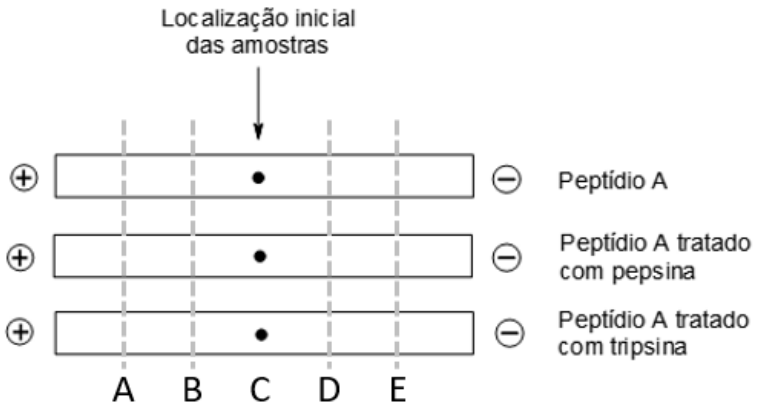
Peptídeo A: H_3N^+-Glu-Leu-Phe-Arg-Gly-Pro-Lys-Leu-Tyr-Ala-Ile-COO^-

i) Pepsin catalyzes the hydrolysis of peptide bonds in which the amino groups of phenylalanine, tyrosine and tryptophan participate.

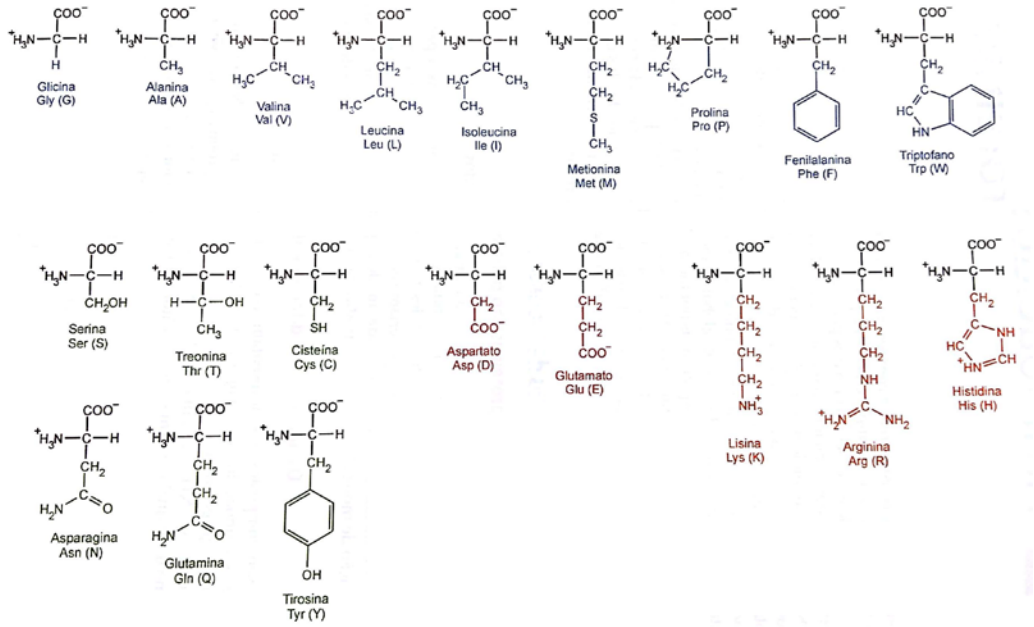
ii) Trypsin catalyzes the hydrolysis of peptide bonds in which arginine and lysine carboxyl groups participate.

iii) The structure of the amino acids and their pKas are shown in Scheme 1 on the last page of the exam.

Based on the above information:
a) Write the peptide sequences resulting from the pepsin-treated samples using the structures shown in scheme 1.
b) Write down the peptide sequences resulting from the trypsin-treated samples.
c) Write, next to each peptide, its charge at pH 7.0.
d) Indicate in the diagram below in which positions (A to E) would you expect to find the results of the electrophoresis carried out at pH 7.0 of a) peptide A, b) peptide A treated with pepsin and c) peptide A treated with trypsin? Indicate the composition of the spot found in each position.

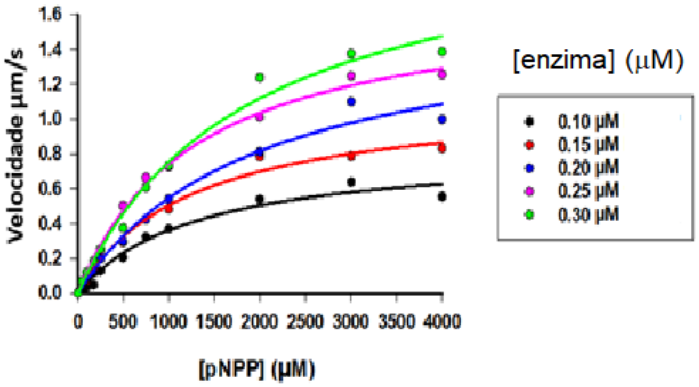


Annexes to Question 1



Aminoácidos livres	pK_1 (α– COO^-)	pK_2 (α– NH_3^+)	pK_R (grupo R)
Glicina	2,35	9,78	
Alanina	2,35	9,87	
Valina	2,29	9,74	
Leucina	2,33	9,74	
Isoleucina	2,32	9,76	
Metionina	2,13	9,28	
Prolina	1,95	10,64	
Fenilalanina	2,20	9,31	
Triptofano	2,46	9,41	
Serina	2,19	9,21	
Treonina	2,09	9,10	
Asparagina	2,14	8,72	
Glutamina	2,17	9,13	
Cisteína	1,92	10,70	8,37
Tirosina	2,20	9,21	10,46
Lisina	2,16	9,06	10,54
Arginina	1,82	8,99	12,48
Histidina	1,80	9,33	6,04
Aspartato	1,99	9,90	3,90
Glutamato	2,10	9,47	4,07

Q2. In one experiment, tyrosine phosphatase enzyme activity was measured at different enzyme concentrations (graph legend) and different p-Nitrophenyl-phosphate (pNPP) substrate concentrations, obtaining the results in the figure below.



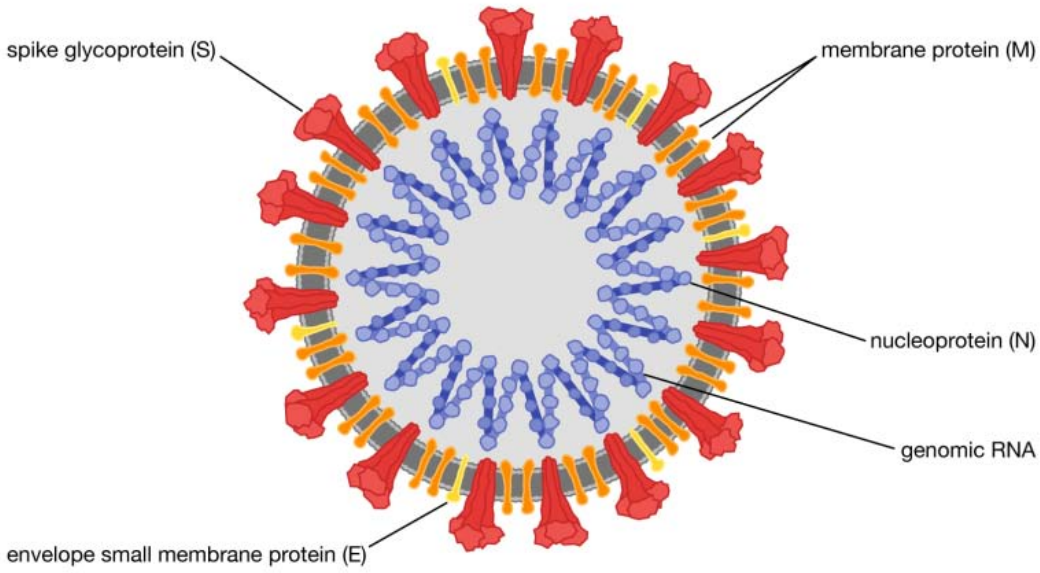
a) Fill in the table below with the requested information (approximate values). Explain how you arrived at the results.

b) Does kcat increase or remain constant with increasing enzyme concentration? Explain.

[E]	$V_{máx}$	K_M	k_{cat}
0.10			
0.15			
0.20			
0.30			

Q3. The SARS-CoV-2 virus has a well-known structure. It is an RNA coronavirus that has a lipid bilayer, the envelope, and four structural proteins: N (viral nucleocapsid), M (membrane protein), S (spike glycoprotein), and E (envelope protein).
Protein N surrounds the virus genome. The M protein is essential for virus stability and assembly. Protein S is responsible for binding to the polar portion of host cell receptors and initiating their invasion. Protein E forms an ion channel in the virus membrane. Proteins M, E and S, are linked to the lipid bilayer.

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)



Considering the information above, answer:

a) Does the virus have genetic material? Justify.

b) How can the virus's lipid bilayer help it infect a new cell?

c) What structural features (primary, secondary, tertiary and quaternary structure) would be expected in E protein? Justify.

d) What structural features (primary, secondary, tertiary and quaternary structure) would be expected in protein S? Justify.

Q4. Aiming to understand the metabolic functioning of muscle cells, researchers performed in vitro experiments using cell cultures, adding the substrates glucose or palmitate (16-carbon fatty acid) in the presence and absence of some compounds (K or M). All experiments were carried out in aerobiosis, and, in the end, some products were detected. The results obtained are shown in Table 1 below. The symbol (+) indicates the formation of the product and (-) the non-detection of the product. Some helpful information can be found in Table 2.

Tabel 1

Experiment	Substrate	[Lactate]	[Acetyl-CoA]	[CO_2]	[ATP]
1	Glucose	-	+	++	++
2	Palmitate	-	+	+++	+++
3	Glucose + **K**	+++	-	-	+
4	Palmitate +**M**	-	+++	-	-

a) In experiments 1 and 2, by what pathways were glucose and palmitate oxidized to CO2? Where do they occur in the cell?
b) What compounds could be K and M?
c) In experiment 3 if compound K were Fructose 2,6-bisphosphate, what would be the expected result in the table?
d) In experiment 4, if compound M were L-carnitine, what would be the expected result in the table?

Tabel 2

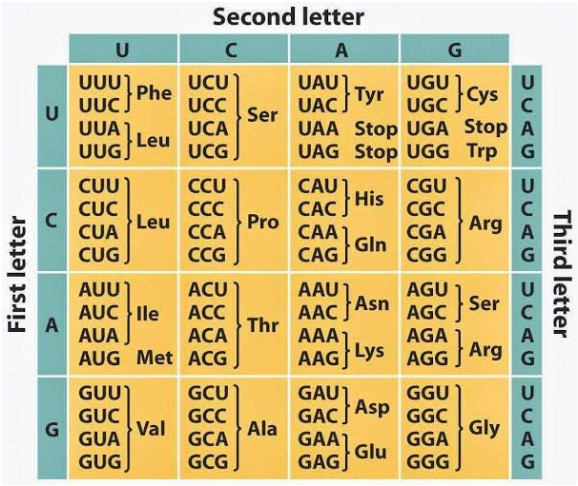
Enzyme	**Allosteric effector**	
	Positive	**Negative**
Phosphofructokinase 1	Fructose 2,6 bisphosphate	ATP, citrate
Frutose 1,6 bisfosfatase		Fructose 2,6 bisphosphate
Isocitrate dehydrogenase	ADP	NADH
Pyruvate carboxylase	Acetyl-CoA	

Q5. When oxygen is added to an anaerobic suspension of cells consuming glucose at a high rate, that rate dramatically decreases as oxygen is consumed and lactate build-up ceases.
a) Why does lactate build-up cease after oxygen is added?
b) Why does the presence of oxygen decrease the rate of glucose consumption?

Q6. Based on the list of codons and amino acids below, which of the following statements are correct? Justify each item.

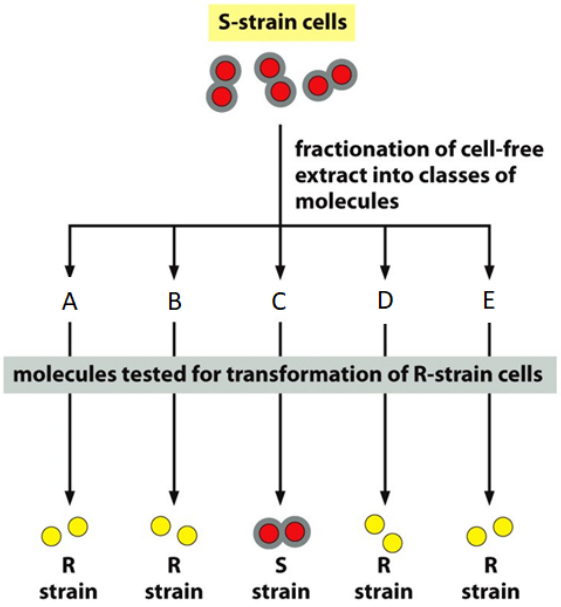
AGU = serine	AGC = serine
AAU = asparagine	AAC = asparagine
AUG = methionine	AUA = isoleucine

a) the genetic code is degenerate.
b) the alteration of a single nucleotide in the DNA that directs the synthesis of these codons could lead to serine substitution for asparagine in the polypeptide.
c) the alteration of a single nucleotide in the DNA that directs the synthesis of these codons would necessarily lead to the substitution of an amino acid in the encoded polypeptide.
d) a tRNA with the anticodon ACU would bind to a ribosome in the presence of one of these codons.



First letter	Second letter: U	Second letter: C	Second letter: A	Second letter: G	Third letter
U	UUU, UUC: Phe; UUA, UUG: Leu	UCU, UCC, UCA, UCG: Ser	UAU, UAC: Tyr; UAA: Stop; UAG: Stop	UGU, UGC: Cys; UGA: Stop; UGG: Trp	U C A G
C	CUU, CUC, CUA, CUG: Leu	CCU, CCC, CCA, CCG: Pro	CAU, CAC: His; CAA, CAG: Gln	CGU, CGC, CGA, CGG: Arg	U C A G
A	AUU, AUC, AUA: Ile; AUG: Met	ACU, ACC, ACA, ACG: Thr	AAU, AAC: Asn; AAA, AAG: Lys	AGU, AGC: Ser; AGA, AGG: Arg	U C A G
G	GUU, GUC, GUA, GUG: Val	GCU, GCC, GCA, GCG: Ala	GAU, GAC: Asp; GAA, GAG: Glu	GGU, GGC, GGA, GGG: Gly	U C A G

Q7. The scheme below summarizes the experiment performed by Avery, McLeod and McCarthy in 1944. In this experiment, an extract of heat-killed virulent Pneumococcus bacteria (strain S) was fractionated into different molecular components (A - E), and each of these purified components was added to non-virulent Pneumococcus bacteria (R strain). The resulting strains were tested for virulence when injected into mice.



a) Indicate the correct option:

i) A= protein; B=DNA; C= lipids; D=RNA; E=carbohydrates
ii) A= proteins; B=DNA; C=RNA; D= lipids; E=carbohydrates
iii) A= protein; B=RNA; C=DNA; D= lipids; E=carbohydrates
iv) A= carbohydrates; B=DNA; C=RNA; D=lipids; E= protein
v) A= carbohydrates; B=RNA; C= proteins; D=DNA; E= lipids

b) What was the conclusion of the experiment?
c) Describe a recombinant DNA technology that is based on the results of this experiment.