

Directions: Please answer all questions only in the space provided.

1. The internal pH of a muscle cell is 6.8. Phosphate in the muscle cell, 10 mM, can exist in 4 forms; the pKa's are 2.15, 6.78, 12.4. At the pH of a muscle cell, how much of each form of phosphate (H_3PO_4 , H_2PO_4^- , HPO_4^{2-} and PO_4^{3-}) is present? You need to calculate this for each form, some will be present at a very much higher concentration than others. (4 points)

2. Diagram all atoms of tryptophan as the butyl ester of N-acetyl-D-tryptophan. (2 points).

3. Diagram the ionic form of cysteine having a charge of -1. (2 points).

4. You have a 50 ml solution of 25 mM histidine at pH 0. How many equivalents of NaOH does it take to titrate histidine to pH 12.5? (3 points).

5. A small octapeptide was isolated from Egret brain. Amino acid analysis from 6N HCl hydrolysis indicated: 14 μ moles alanine, 17 μ moles ammonium, 15 μ moles valine, 16 μ moles arginine, 46 μ moles of isoleucine, 15 μ moles of glycine, 17 μ moles of aspartate.

a. in one molecule of the octapeptide, how many residues are present of each amino acid. **Use only the one-letter code.** (2 points)

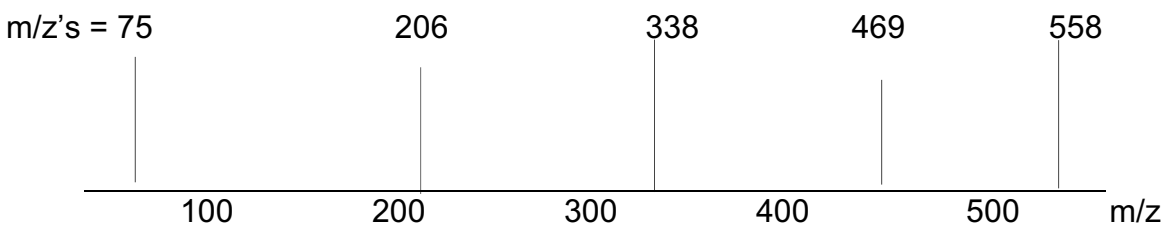
b. Chemical treatment of the free carboxyl followed by 6N HCl hydrolysis yielded 2-amino-propanol. What does this say about the octapeptide? (2 points)

c. Treatment with dinitrofluorobenzene resulted in the production of a dinitrophenyl-amino acid that had 5 carbons. What does this say about the peptide? (2 points)

d. Treatment of the peptide with trypsin resulted in a pentapeptide and a tripeptide containing the N-terminal amino acid. Draw the primary structure of the tripeptide using for unknown residues at this point (such as T P could be TAP or TLP, etc.) (2 points)

e. The Edman degradation was performed through two cycles (rounds) on the pentapeptide. It produce the hydantoin derivative of aminoacetic acid on round 1, the hydantoin of isoleucine in round 2. Using single letters and underlined blank spots for not yet determined amino acids, what is the primary sequence of the pentapeptide? (2 points).

f. The pentapeptide was used to inject a tandem electrospray mass spectrometer. The following spectrum was obtained:



What is the primary sequence of the complete octapeptide? (3 points)

6. The following table was made from data obtained during the purification of xanthine dehydrogenase from a fungus culture.

Fraction	Volume (ml)	Total Protein (mg)	Activity ($\mu\text{M}/\text{sec}$)
Crude Extract	3,800	22,800	2,460
$(\text{NH}_4)_2\text{SO}_4$ ppt	165	2,800	1,190
Ion Exchange Chromotography	65	100	720
Affinity Chromotography	6	1.8	555

a. What are the specific activities of the crude extract and final product? (2 points)

b. Which step in the fractionation produced the greatest purification? (2 points)

c. What is the final fold purification? And, how would you determine if the protein was pure? (3 points)

7. You discovered in the last exam that Blue Heron and Alligator glucokinase had the same V_{max} (0.2 mM/min) but they had different K_M 's. Blue Heron had a $K_M = 0.67$ mM and Alligator had a $K_M = 0.33$ mM. Assume that these assays were done with equivalent amounts of enzyme. Which enzyme has the greatest efficiency? Explain. (4 points)