

1. 50 Points. Amino Acid Sequence Problem

A peptide had the following amino acid composition obtained by acid hydrolysis and amino acid analysis: Arg, Ala, Gly, Glu, Leu, Lys, Pro, Ser, Tyr

The peptide was processed by the Dansyl-chloride method and Dansyl-Ala was found.

Next the peptide was treated with trypsin which gave 2 peptides, called T-1 and T-2. T-1 was a tetrapeptide with amino acid composition: Tyr, Glu, Ser, Gly. Edman degradation yielded Gly as first amino acid released and then Tyr. T-2 was a pentapeptide with amino acid composition of Lys, Pro, Ala, Arg, Leu. Edman degradation yielded Ala first, then Leu, then Arg.

Chymotrypsin treatment of the peptide gave 2 peptides, called C-1 and C-2. C-1 had amino acid composition (acid hydrolysis): Ala, Tyr, Gly, Arg, Lys, Pro, Leu. Edman degradation of C-1 gave Ala in 1st cycle, Leu in 2nd cycle and Arg in 3rd cycle. C-2 had amino acid composition (acid hydrolysis): Ser, Glu. Edman degradation of C-2 gave Ser in 1st cycle and the amino acid Glu was remaining.

What is the sequence of the peptide?

Draw the full chemical structure of the peptide at pH 7.

What is the net charge on the peptide at pH 1, 5, 7, 10, 13?

What is the pI?

pK values for amino acids: Gly - 2.4, 9.8; Ala - 2.4, 9.9; Tyr - 2.2, 9.1; Pro - 2.0, 10.7; Glu - 2.1, 4.1, 9.5; Gln 2.2, 9.1; Arg 1.8, 9.0, 12.5; Lys 2.2, 9.2, 10.8; Ser 2.2, 9.2; Leu 2.3, 9.7

2. 25 Points - Cell Structure and Function (5 points each short answer)

- What is an enzyme and what does it do in the cell?
- How many amino acids are encoded by the genetic code? Could there be more? Why not more?
- What is the difference between DNA and RNA in chemical structure (Deoxyribo-Nucleic Acid versus Ribo-Nucleic Acid) and in biological function?
- What advantage does a eukaryotic cell gain by having organelles like mitochondria, nucleus, etc?
- What do green plants do for other organisms on earth (be brief but complete)?

3. 15 Points - Protein Purification (5 points each short answer)

(Explain your answer briefly but fully)

- What is Native PAGE and what is its purpose?
- What SDS-PAGE (denaturing PAGE) and what is its purpose?
- What is the advantage of affinity chromatography over other purification methods like ion exchange chromatography or gel filtration?

4. 10 Points Thought Question

List at least 5 proteins that you know and provide an explanation of the cellular/organism function of the proteins. Identify them as specifically as you can in 3 words or less (example - human hemoglobin) and then explain in a few words the function of the protein (example – hemoglobin transports oxygen in the blood).

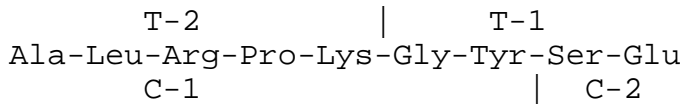
ANSWERS

1. 50 Points. Amino Acid Sequence Problem

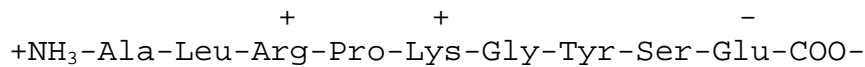
Sequence of the peptide is:

AlaLeuArgProLysGlyTyrSerGlu

The Trypsin and Chymotrypsin cleavage sites and peptides (T-1 & T-2; C-1 & C-2) are:



The chemical structure can be in Lecture 4 for amino acids and Lecture 5 for peptides. The charges on the N- and C-termini and ionizable side chains for Arg, Lys and Glu at pH 7 are as shown below:



The Net Charges at various pH values are:

| | | | | | |
|-------------|----|----|----|----|----|
| pH | 1 | 5 | 7 | 10 | 13 |
| Net Charge: | +3 | +1 | +1 | 0 | -2 |

The pI = 10.35 or 10.4

This is because the peptide at pH 7 has net charge of +1 so to get to the "0" form of the peptide you need to remove the proton from the alpha-amino group which has a pK of 9.9; then to get to -1 net charge you need to remove the epsilon-amino group (side chain) of Lys which has a pK of 10.8. So you average 9.9 + 10.8 to obtain the pI of 10.35.

2. 25 Points - Cell Structure and Function (5 points each short answer)

A. What is an enzyme and what does it do in the cell?

An enzyme is a protein which serves the cell as a bio-catalyst.

B. How many amino acids are encoded by the genetic code? Could there be more? Why not more?

There are 20 amino acids encoded by genetic code. Yes there could be more, even as many as 61 to 63, but there seems to be no need to encode more than 20 amino acids since this provides enough diversity in structural and functional types to build a huge variety of proteins and enzymes.

C. What is the difference between DNA and RNA in chemical structure (Deoxyribo-Nucleic Acid versus Ribo-Nucleic Acid) and in biological function?

DNA has the 2' hydroxyl group of the ribose sugar reduced to a hydrogen and RNA does not, which makes RNA less stable than DNA. Since DNA is the long term storage polymer for keeping genetic information, it needs to be more stable than RNA which serves the cell transient roles like mRNA. There are also some differences in the aromatic bases in DNA and RNA also.

D. What advantage does a eukaryotic cell gain by having organelles like mitochondria, nucleus, etc? The organelles serve specific functions but also provide unique environments within the cell for separating processes like synthesis and degradation of metabolites. In addition, the organelles permit the cell to have greater control over the more complex metabolism that is found in eukaryotic cells.

E. What do green plants do for other organisms on earth (be brief but complete)?

Green plants convert sunlight energy via photosynthesis to provide the energy for carbohydrate production from CO₂ and oxygen is also produced as a by-product. From a modern point-of-view, plants collect the “greenhouse gas” carbon dioxide which may help keep the planet cooler, while producing oxygen which is not a greenhouse gas.

3. 15 Points - Protein Purification (5 points each short answer)

A. What is Native PAGE and what is its purpose?

Native PAGE is polyacrylamide gel electrophoresis where the proteins are separated under conditions where native functionality is retained. Native PAGE is used to determine the purity of an enzyme preparation where the resulting gel can be stained for both enzyme activity and protein content. In Native PAGE, if a single protein band is found which has the enzyme activity then the protein is homogeneous.

What SDS-PAGE (denaturing PAGE) and what is its purpose?

SDS PAGE is polyacrylamide gel electrophoresis under denaturing conditions which is done to determine the molecular weight of the polypeptide subunit of a protein. It can also serve the purpose of determining if a protein is pure or not, but only if one already knows that it does not have more than one polypeptide chain in its native structure.

What is the advantage of affinity chromatography over other purification methods like ion exchange chromatography or gel filtration?

Affinity chromatography, which is based on the special characteristics of the protein involved with its biological functionality, is more specific than either ion exchange chromatography or gel filtration, which are both based on general features of all proteins such as net charge at a specific pH (ion exchange) and differences in molecular size of the proteins (gel filtration).

4. 10 Points Thought Question

List at least 5 proteins that you know and provide an explanation of the cellular/organism function of the proteins. Identify them as specifically as you can in 3 words or less (example - human hemoglobin) and then explain in a few words the function of the protein (example – hemoglobin transports oxygen in the blood).

Your choice here: there are thousands of proteins to select from and most have known functions.