

1. 50 Points. Amino Acid Sequence Problem

A peptide had the following amino acid composition obtained by acid hydrolysis and amino acid analysis: Ala, Arg, His, 2 Glu, Gly, Lys, Met, Pro, Tyr. N-terminal analysis of the peptide yielded Dansyl-Met. C-terminal analysis with carboxypeptidase showed Ala was released 1st.

When the peptide was treated with cyanogen bromide (CNBr), an amino acid with strange properties and a peptide with 9 amino acids were obtained after hydrolysis with water. When analyzed with the Edman method, the peptide released Gln first, then Glu, then Tyr.

Treatment of the original peptide with chymotrypsin yield 2 peptides, Ch-1 and Ch-2. Ch-1 analyzed by Edman degradation yielded Arg, then Pro, then Lys. Edman on Ch-2 yielded Met, then Gln, then Glu.

Treatment of the original peptide with trypsin yield 2 peptides, T-1 and T-2. T-2 yielded in Edman degradation His 1st, Gly 2nd and Ala was left over.

What is the sequence of the peptide?

Draw the full chemical structure of the peptide at pH 7 with all atoms shown including hydrogens.

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; His 1.8, 6.0, 9.3; Met 2.1, 9.3

2. 20 Points - Cell Structure and Function (4 points each short answer)

- List some functions that proteins carry out in living cells? List of 4 functions needed for full credit.
- Explain how protein synthesis works in a prokaryotic cell, include in your answer the processes of transcription and translation and the nature of the universal genetic code used by living systems. (The use of diagrams in your answer is strongly encouraged).
- A current experimental method for trying to cure disease in humans is gene replacement therapy – explain the underlying biochemical principles being used in this medical treatment.
- Explain why Dolly (the “cloned” sheep) is not an exact genetic replica of the ewe (Dolly’s “mother”) that donated the nucleus for the cloning process used to generate Dolly.
- During evolution of living systems, how was the earth and its atmosphere changed by the advent of green plants and their domination of the earth for millions of years **and** how did this impact the biochemical possibilities for living systems?

3. 20 Points - Protein Purification (5 points each short answer)

(Explain your answer briefly but fully – use diagrams where ever possible)

- How do you determine if a protein is pure after a series of purification steps have been done?
- How do you denature a protein for SDS-PAGE and determine the molecular weight of its polypeptide chain (assume there is only 1 polypeptide chain in the protein)?
- Gel filtration is both a purification tool and biochemical characterization method for proteins, explain how it works and what quantitative information you can obtain about a protein from using it (include how you get the quantitative information).
- How do you do Affinity Chromatography for purification of protein? What property of the protein do you take advantage of when you make a support gel with ligand for Affinity Chromatography?

4. 10 Points Thought Question

Why is it advantageous for living systems to have both aqueous and lipid/organic phases in their cells?

amino acids and energy for the synthesis of the peptide bond. Additional energy, which is supplied as GTP, is needed to enhance the efficiency of the translation process and drive the movement of the m-RNA relative to the ribosomal binding sites for the growing peptide and the incoming t-RNA/AA. The net result of protein synthesis done in this manner is that the protein is a linear replica of the coding sequence in the DNA, such that protein sequences can be deduced from DNA sequences. Diagrams illustrating protein synthesis are in Lecture 2 and the genetic code was presented in Lecture 4 on the structure of the 20 amino acids found in proteins and encoded by genetic code. Eukaryotic protein synthesis differs in that a pre-mRNA is made from the DNA which must be processed to remove intervening “intron” nucleotide sequences before the m-RNA can be translated.

- C. In gene replacement therapy, the disease is sometimes caused by a defective protein due a mutation in the person’s DNA. Thus, the concept is to take precursor cells for the effected tissue and incorporate, using recombinant DNA technology, a non-mutant copy of the gene causing the problem. Then to plant the genetically modified cells in the person’s bone marrow which may then synthesize the correct protein from the replacement gene and cure the disease as the cells for the effected tissue are replaced by the descendants of the implanted, modified precursor cells. This has not yet worked well in humans.
- D. Dolly got her mitochondria from the sheep cells used in the cloning process and not from the ewe (her “mother”) donating the nucleus and so Dolly’s extra-nuclear or cytosolic DNA in her mitochondria is not the same as the ewe who gave the nuclear DNA. Thus, Dolly is a clone or exact genetic replica of the ewe only with respect to the nuclear genes and not the mitochondrial ones.
- E. Green plants are autotrophic (capacity to make all their own energy and biochemical building blocks) and generate stored carbon and nitrogen used by animals for food as well as the oxygen “breathed” by all aerobic organisms. So when plants took over the earth millions of years ago, huge quantities of oxygen were released and a new atmosphere formed on earth and huge amounts of carbon were accumulated in the forms of coal, oil and methane gas as the plants died and became incorporated in the earth’s crust. This of course opened up the possibility of aerobic biochemistry on earth which resulted in a new type of organisms dependent on oxygen as an electron acceptor and usually also dependent on other organisms to supply biochemical building blocks and energy molecules.

3. 20 Points - Protein Purification (5 points each short answer)

See Lectures 6 & 7 for the diagrams to go with the answers to these questions.

- A. Purity of a protein is determined by native polyacrylamide gel electrophoresis (PAGE) where you must show that a single protein band is obtained for the homogeneous protein when the gel is stained for protein and demonstrate that this protein band displays the biochemical property expected for your protein of interest – ie. Enzyme activity using a specific stain for your enzyme or other property such as red color for hemoglobin with its characteristic light absorbance spectrum.
- B. A protein is denatured for SDS-PAGE by heating the protein in presence of 2-mercaptoethanol to reduce its disulfide bonds and the detergent SDS, which coats the backbone of the protein giving it a negative charge in proportion to its size and keeping it in solution in the linear, denatured form. The molecular weight of the denatured polypeptide is determined by SDS-PAGE using calibration with standard proteins of known molecular weight for their polypeptide subunits.
- C. Gel filtration is a reverse sieving process where proteins are separated by size via synthetic beads with molecular size holes that allow smaller proteins to enter the beads when larger ones are excluded. One can obtain a measure of the native molecular weight for a protein, which can be combined with SDS-PAGE analysis of its subunit size to determine the subunit composition of the protein. For example, a protein with a native molecular weight of 49,000 and a subunit size for its polypeptide of 24,000 is a dimer – ie. $49,000/24,000 = \sim 2$.
- D. Affinity Chromatography takes advantage of a protein’s biological activity to purify the protein. For example, an enzyme has a specific active site where it binds its substrate. Inhibitors that chemically look like the substrate and bind with high affinity at the active site can be used as ligands to bind the

enzyme to a gel which has been covalently modified to display the inhibitor to the solution on the surfaces of the gel beads. The crude mixture of proteins containing the one you want to purify is poured on the affinity gel and washed until the non-binding proteins are removed and then the bound protein of interest is eluted by applying the substrate. The substrate can be removed by gel filtration.

4. 10 Points Thought Question

Non-aqueous phases or lipid/organic phases in cells in the form of membranes provide limiting barriers relatively impermeable to water, which allow the main aqueous phase of the cell, the cytosol, to be maintained intact and, in fact, membranes create the cell as a water-containing structural unit. Other membrane systems in the cell create other environments such as the “matrix of the mitochondria” which is the aqueous phase in this organelle where most of the ATP is synthesized in a cell.

Aqueous and non-aqueous or lipid/organic phases in a cell provide two different chemical environments for the synthesis of the two types of molecules needed by the cell: those that are water soluble such as ionic components like proteins and metabolites like phosphorylated sugar molecules; and those that are water insoluble and must be maintained in a lipid environment such as cholesterol which is always in a membrane or bound to a carrier protein or when it is in excess it may accumulate as insoluble layers in your blood vessels like in arteriosclerosis or hardening of the arteries.

To get the full ten points for this question you must cover both aspects of cell biochemistry as described above. So if you say something about membranes and nothing about biosynthetic environments, then you get 5 points. Or vice-versa, if you say need 2 environments because you need both water soluble and insoluble components in cells and do not mention membranes, then you get 5 points. The number of points awarded also varies depending on the quality of your answers – ie. If you give a really “good” answer for one the parts you may get a bit more than 5 points or if you just say, “membranes” and do not explain what they do for the cell then you may have gotten fewer than 5 points for one of the aspects. In some cases, if you give a really “great” answer for one part, you might have gotten 10 points!

General Comment on Grading Thought Questions: Since Thought Questions are not testing you on information I specifically provided in a lecture, I seek to test your general knowledge of biochemistry by asking you to draw on your overall education and experience so far in your life. Since there is no specific lecture material directly related to Thought Questions, you can not study to prepare yourself to answer these questions, which some students find very frustrating. Grading Thought Questions is based on my subjective views and the points awarded are all judgment calls on my part and I can not provide any greater insight for you as to how I decide the points for Thought Questions. However, I am looking for you to show me that you can think about biochemistry and write clear explanations of biochemical principles in Thought Questions. The impact of Thought Questions on your final letter grade in this course is usually small unless you are at or near the top end of the grading scale. However, it is usually possible to get an “A” in this course even if you never score a point on the Thought Questions!

NOTE: The score you got on Exam I may be challenged by seeing me. I make mistakes both in adding up scores and giving points. So please see me if you feel your exam was not graded correctly. However, please note, I allow only the next 2 weeks for these corrections to be made. Thus, if you want to have me look over your exam for mistakes, then you must see me by Friday, October 15th by the end of the day at 5 PM. I will announce this in class to be sure everyone is aware of the deadline for Exam I score revisions. Do Not Be Shy – if you think you deserve more points, be sure to see me before or on Oct 15th.