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PROTEOMICS

9815/01

Paper 1

24 September 2014

2 hour 30 minutes

Additional materials:

Answer Paper

Graph Paper

Appendix List of Amino Acids (with Question Paper)

READ THESE INSTRUCTIONS FIRST

Write your index number, CT group & name on all the work you hand in.

Write in dark blue or black pen on both sides of the paper.

You may use a soft pencil for any diagrams, graphs or rough working.

Do not use staples, paper clips, highlighters, glue or correction fluid.

Section A

Answer **all** questions.

Section B

Answer **three** out of **four** questions.

Section C

Answer the question

At the end of the examination, fasten all your work securely together. **Please hand in this question booklet together with your answer sheets.**

The number of marks is given in brackets [] at the end of each question or part question.

You may use a calculator.

You are reminded for the need for clear presentation in your answers.

This document consists of **8** printed pages.



Section A

Answer all the questions in this section.

- 1 A hydrophobicity plot shows the distribution of polar and non-polar residues along a protein sequence. Amino acid polarity is usually expressed as a number known as hydrophathy index, where the amino acids are ranked along a hydrophobicity scale. Fig. 1.1 shows the hydrophobicity plot of bacteriorhodopsin, a membrane protein.

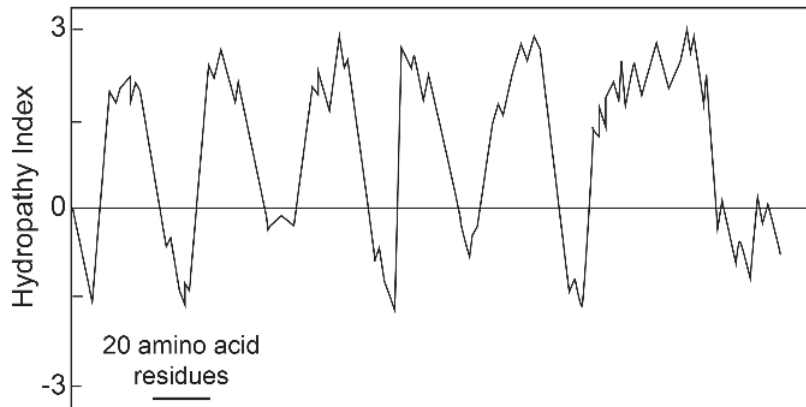


Fig. 1.1

- (a) Cell membranes are approximately 30\AA thick. With reference to Fig. 1.1, predict the number of membrane-spanning alpha helices in bacteriorhodopsin. Explain your answer. [4]
- (b) (i) Further studies on the amino acid sequence of bacteriorhodopsin reveal that it contains many amino acid residues with ionisable R-groups within the membrane-spanning regions. Suggest what type of membrane protein, bacteriorhodopsin is. [1]
- (ii) Name one other example of a membrane protein that is of the same type as the one stated in (b)(i). Briefly describe its biological role. [3]
- (c) One of the membrane-spanning alpha helices is displayed on a helical wheel diagram as shown in Fig. 1.2. This segment consists of 18 amino acids with tryptophan as the first amino acid residue of the alpha helix.

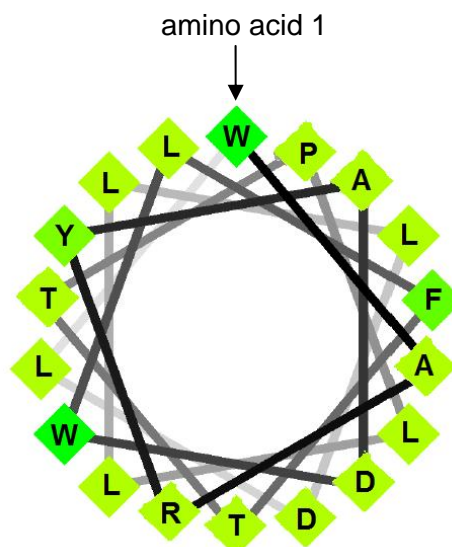


Fig. 1.2

With reference to Fig. 1.2,

(i) write down the amino acid sequence of this segment. [1]

(ii) explain what structural information can be concluded from the helical wheel. [2]

(d) Discuss the limitations of studying amino acid sequences. [2]

[Total: 13]

2 Great strides have been made in the past sixty years in understanding the biosynthesis of purines. A putative multi-protein complex called the purinosome, is amongst the most recent discoveries relating to purine biosynthesis.

(a) The purinosome is found to contain six different enzymes involved in the biosynthesis of purines. Discuss the advantages of such a macromolecular complex. [2]

(b) Research that attempts to characterize the purinosome *in vitro* has been challenging. Suggest why it is difficult to perform *in vitro* research on the structure of this macromolecular complex. [2]

(c) Using a specific example, discuss how coupling with cofactor resulting in alteration in function is achieved in multi-enzyme complexes. [4]

(d) Many other proteins are homomultimers. Discuss the advantages of homomultimers. [3]

[Total: 11]

3 A large variety of proteins including blood coagulation factors are synthesised as inactive precursors called pre-pro-proteins. These inactive precursors must undergo post-translational processing to become biologically active polypeptides.

(a) The N-terminal pre-regions are signal peptides which direct the proteins to a specific location in the cell. State the location within the cell where such proteins are directed to. Explain why it is important for proteins to go to this part of the cell. [4]

(b) Proprotein convertases act on proproteins to form biologically active proteins. Name a specific proprotein, and discuss how active proteins are produced. [3]

(c) Discuss how targeting proteins for degradation can help regulate reactions in a cell. [2]

[Total: 9]

- 4 Fig 4.1 below shows a diagram of human immunoglobulin G (IgG) raised against the virus, influenza A/Puerto Rico/8. This IgG is labeled anti-PR8.

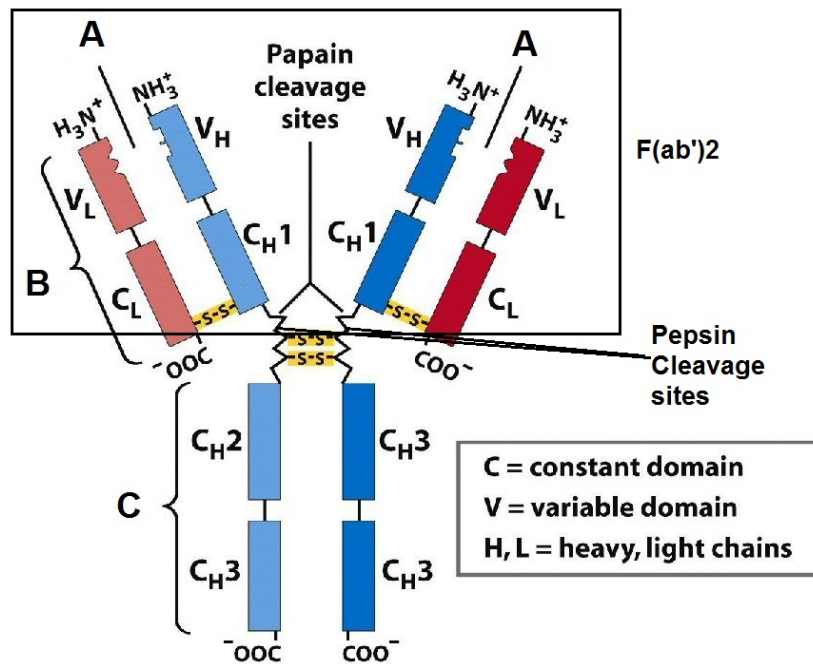


Fig 4.1

- (a) With reference to Fig 4.1, identify structures A and C. [2]
- (b) The anti-PR8 IgG antibodies were purified using affinity chromatography. A fraction of the anti-PR8 was treated with pepsin and anti-PR8 F(ab')₂ was harvested. The anti-PR8 IgG and F(ab')₂ were then analysed using both reducing and non-reducing SDS PAGE.

Fig 4.2 shows a diagram of the gel.

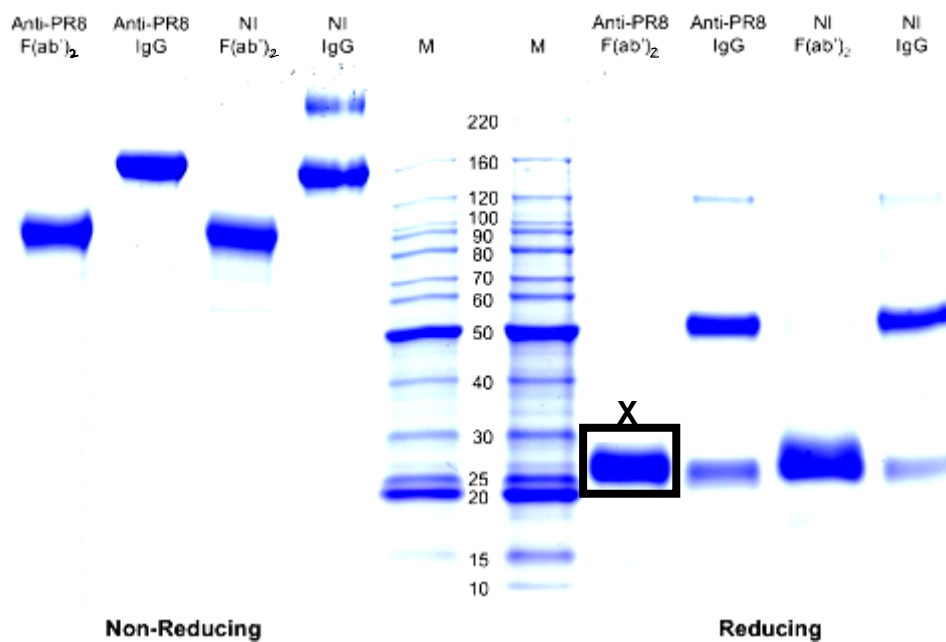


Fig 4.2

- (c) Describe how non-reducing SDS-PAGE is used to separate anti-PR8. [4]
 - (d) Using Fig 4.2 and information provided about the molecular weight marker, show how the molecular size of the band X can be estimated. (Please use the graph paper provided in your estimation) [2]
 - (e) With reference to Fig 4.2, explain the difference in the banding pattern when anti-PR8 F(ab')₂ was separated using a reducing and non-reducing gel. [2]
- [Total: 10]

5 Transcription is controlled by proteins that bind to specific regulatory sequences and modulate the activity of RNA polymerase.

- (a) Transcriptional regulatory proteins in eukaryotic cells can be classified based on their structural motifs and four families of motifs are especially common. Name two of these motifs and describe their structures. [4]
 - (b) Eukaryotic transcription is far more complex than bacterial transcription. The main difference lies in the RNA polymerase. Discuss this difference. [3]
- [Total: 7]

Section B

Answer 3 out of the 4 questions in this section.

- 6** Elmo-1 is an evolutionarily conserved mammalian protein that is known to have roles during the removal of apoptotic cells, cell migration, neurite outgrowth, and myoblast fusion. Elmo-1 mediates these cellular processes by interacting with various proteins located in the plasma membrane, cytoplasm and nucleus.
- (a) Discuss how yeast-2-hybrid is used to identify interacting partners of Elmo-1. [4]
 - (b) Among all the clones screened, Arhgef16 was found to interact with Elmo-1. Describe the subsequent steps taken to determine the identity of Arhgef16 and its putative function. [4]
 - (c) Describe an *in vitro* method that can be used to verify protein interaction between Elmo-1 and the protein encoded by Arhgef16. [2]
- [Total: 10]
- 7**
- (a) Name a kinase and explain why it is essential to the normal functioning of a cell. [5]
 - (b) Describe terminal acetylation and discuss the effect that this may have on a protein. [3]
 - (c) The enzyme lysozyme hydrolyses peptidoglycan. The peptidoglycan molecules are hydrolysed at rates of up to 0.5 mol per active site per second. A competitive inhibitor of lysozyme, called (NAG)₃ is hydrolysed far more slowly, at a rate of just 8×10^{-6} mol per active site per second.
- Suggest why (NAG)₃ yields far more information than peptidoglycan about enzyme-substrate interactions when investigating how the lysozyme substrate is hydrolysed using X-Ray crystallography. [2]
- [Total: 10]
- 8**
- (a) Explain how two non-covalent interactions are important to the function of haemoglobin. [4]
 - (b) With reference to Christian Anfinsen's experiment using ribonuclease, discuss the major factor that maintains the native structure of a protein and its function. [3]
 - (c) What is SDS and briefly explain the difference in how SDS and β -mercaptoethanol cause protein denaturation. [3]
- [Total: 10]
- 9**
- (a) Name and describe two types of protein-protein binding. [4]
 - (b) Discuss how ligand binding brings about allosteric changes in enzymes. [4]
 - (c) Distinguish between a protein's binding cleft with the core of a folded polypeptide. [2]
- [Total: 10]

Section C

Answer the question in this section.

- 10** Immunomics is the study of immune system regulation and response to pathogens using genome-wide approaches. Traditionally, researchers have to search for antigens and identify the protein sequence of these antigens that is recognized by the immune system.
- (a) Name the part of an antigen that is recognized by an antibody and describe one feature of an antigen-antibody interaction. [3]
 - (b) Discuss the use of antibodies in research and diagnostics. [7]
 - (c) Cancer is a multi-step process and frequently involves accumulation of multiple gene mutations. The gain-of-function and loss-of-function mutations of crucial genes involved in cell cycle regulation frequently lead to tumour formation.

Hepatocellular carcinoma is the most common type of liver cancer. In an investigation to uncover how genetic mutation leads to changes in the proteome, you are provided with hepatocellular carcinoma tissues and normal liver tissues.

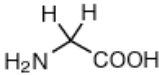
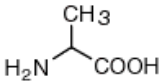
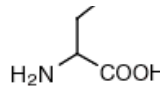
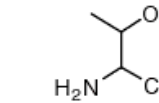
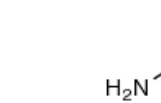
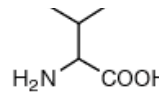
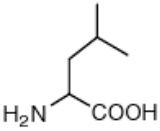
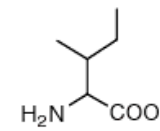
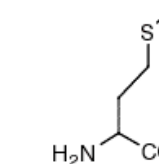
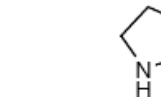
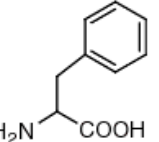
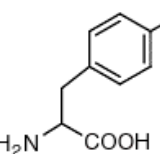
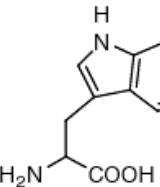
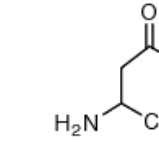
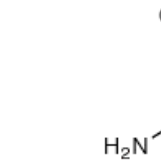
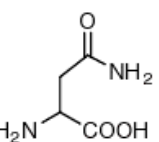
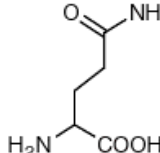
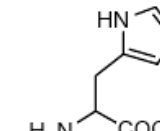
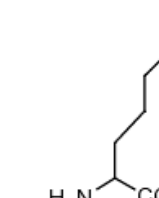

Describe the steps taken to identify changes in the proteome of hepatocellular carcinoma tissues that results in tumour formation. [10]

[Total: 20]

End of Paper

Appendix

List of Amino Acids

 Glycine (Gly, G) MW: 57.05	 Alanine (Ala, A) MW: 71.09	 Serine (Ser, S) MW: 87.08, pK _a ~ 16	 Threonine (Thr, T) MW: 101.11, pK _a ~ 16	 Cysteine (Cys, C) MW: 103.15, pK _a = 8.35
 Valine (Val, V) MW: 99.14	 Leucine (Leu, L) MW: 113.16	 Isoleucine (Ile, I) MW: 113.16	 Methionine (Met, M) MW: 131.19	 Proline (Pro, P) MW: 97.12
 Phenylalanine (Phe, F) MW: 147.18	 Tyrosine (Tyr, Y) MW: 163.18	 Tryptophan (Trp, W) MW: 186.21	 Aspartic Acid (Asp, D) MW: 115.09, pK _a = 3.9	 Glutamic Acid (Glu, E) MW: 129.12, pK _a = 4.07
 Asparagine (Asn, N) MW: 114.11	 Glutamine (Gln, Q) MW: 128.14	 Histidine (His, H) MW: 137.14, pK _a = 6.04	 Lysine (Lys, K) MW: 128.17, pK _a = 10.79	 Arginine (Arg, R) MW: 156.19, pK _a = 12.48