

COPY

THE UNIVERSITY OF MELBOURNE

SEMESTER 1 ASSESSMENT , 2002

DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOLOGY

521-301 – Protein Structure, Design & Engineering

EXAM DURATION: Two (2) Hours & Thirty (30) minutes

READING TIME: Fifteen (15) Minutes

COMMON CONTENT: No

THIS PAPER HAS 5 PAGES

Authorized Materials:

No specific materials are authorized.

Instructions to Invigilators:

Please supply five (5) 6-page examination booklets.

Instructions to Students:

This exam paper consists of FIVE (5) sections

All sections should be attempted

EACH section should be answered in a **SEPARATE** examination booklet.

Use a **pen** and write legibly.

The total number of marks for this examination is **150**.

This examination **equals 80%** of the total marks for this subject.

This paper may be lodged with the Baillieu Library

SECTION I - START A NEW BOOKLET

Question 1

ANSWER EITHER

Describe the use of structure-based protein design strategies to synthesize proteins with modified functional properties. Give examples of the use of this approach.

(25 marks)

OR

- a. Describe the use of combinatorial approaches such as DNA shuffling (*in vitro* recombination) and phage display methods to select for proteins with modified functions.
(20 marks)
- b. Discuss the advantages and disadvantages of structure based design strategies compared to combinatorial methods.
(5 marks)

Question 2

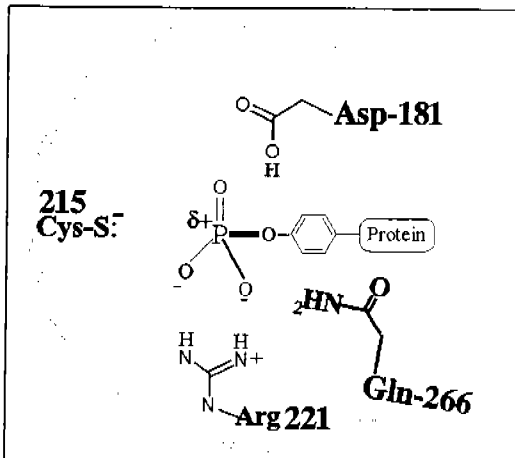
Compare and contrast the roles, structures and mechanisms of action of HSP70 and HSP60 (GroE) proteins. The use of diagrams would facilitate a succinct answer.

(15 marks)

SECTION II - START A NEW BOOKLET

Question 3

Biochemical analyses reveal that the protein tyrosine dephosphorylation reaction catalyzed by all protein tyrosine phosphatases follows a two-step mechanism. In the first step, the phosphate group from the phosphotyrosyl protein substrate is transferred to a catalytically critical residue to form a phosphoenzyme intermediate. In the second step, the phosphoenzyme intermediate is hydrolyzed.



A schematic diagram of the active site of PTP1B depicting the phosphotyrosine-protein substrate and the catalytically critical amino acid residues.

All protein tyrosine phosphatases contain the conserved CX₅R motif and the "WPD" loop in their active site. The diagram schematically shows the active site of PTP1B. In the diagram, residues Cys-215 and Arg-221 correspond to the conserved Cys and Arg residues in the CX₅R motif; Asp-181 corresponds to the conserved Asp in the "WPD" loop. In addition to the CX₅R motif and the "WPD" loop, Gln-266 is also a catalytically critical residue that participates in the second step of the PTP1B-catalyzed dephosphorylation reaction.

Based upon the information provided and your knowledge of the catalytic mechanism of protein tyrosine phosphatases, answer the following questions.

- Use the diagram provided as a guide, schematically draw the chemical mechanism of the two-step dephosphorylation reaction catalyzed by PTP1B. Your drawing should include the structure of the two transition state intermediates, the reaction products, and the phosphoenzyme intermediate.
- Briefly outline the functional roles played by Cys-215, Arg-221 and Asp-181 in the dephosphorylation reaction.
- Compare and contrast the functional roles of Gln-266 in the PTP1B-catalyzed dephosphorylation reaction and those of Gln-61 in the Ras/GAP-catalyzed GTP hydrolysis reaction.

(15 marks)

Question 4

With the help of a schematic diagram, explain how Arg-789 of GAP facilitates the GTP hydrolysis reaction in the active site of Ras.

(10 marks)

SECTION III - START A NEW BOOKLET

Question 5

The interaction of growth hormone with its receptor would be expected to be described by a "bell-shaped" dose response curve. Explain the structural basis for this prediction in terms of (a) the structure of the ligand; (b) the structure of the receptor; (c) the nature of the interactions between the ligand and receptor; then (d) discuss why such curves are not generally observed.

(10 marks)

Question 6

The protein tyrosine kinases that belong to the Tec family (Tec, Btk, Itk, Txk and Bmx) share significant similarity with the Src family of protein tyrosine kinases. However, at their N-termini they have a pleckstrin homology (PH) domain and a Tec homology (TH) region, in place of the Src family SH4 and unique regions. Also, the C-terminal tail of the Tec kinases lack tyrosine residues, but the linkers between the SH2 and kinase domains do contain at least one proline and a conserved bulky hydrophobic residue.

Based on the structural differences listed above, what aspects of Tec kinase regulation would you expect to be (a) the same as Src (b) different from Src? Explain your reasoning.

(15 marks)

SECTION IV - START A NEW BOOKLET

ANSWER EITHER

Question 7

Describe a structural hypothesis of how the Nuclear Receptor Factors may activate transcription. Restrict your answer to a description of structures of the Ligand Binding Domain of these factors.

(25 marks)

OR

Question 8

a. How do the GAL4 family of zinc fingers bind DNA? Describe the structure of the first ~90 residues of the DNA binding domain of GAL4 bound to DNA.

(15 marks)

b. State any similarities and differences in the way GAL4 binds to DNA compared to how the three fingers of Zif268 bind to DNA.

(4 marks)

c. Describe an experiment, other than determining the structure, that resolved what part of the protein is essential for DNA specificity.

(6 marks)

SECTION V - START A NEW BOOKLET

ANSWER EITHER

Question 9

Describe:

- (a) How antibodies can be used therapeutically.
- (b) The production of engineered antibodies that are clinically more useful.

(35 marks)

OR

Question 10

Discuss the basis of peptide binding specificity of MHC class I molecules. Include in your answer the strategy for producing engineered MHC class I-peptide complexes for use as specific T cell probes.

(35 marks)

END OF EXAMINATION