Exam Handout for PHAR2811 students, 2009

Six (6) questions will be asked from this material

You have been given a mixture of 5 proteins (A - E) to separate and identify. You have been told that some of these proteins are coloured, containing a heme prosthetic group, which absorbs strongly at 410 nm. Two of the proteins contain 2 identical subunits (equal molecular weight). You have carried out the following experiments:

**Experiment 1.**
An aliquot of the mixture (A - E) was loaded onto DEAE cellulose at pH 7.0. One mL fractions were collected and the absorbance measured at 410 nm and 280 nm. The first peak eluted contained A and B. A second peak, eluted after the addition of 0.5 M NaCl at pH 7.0 contained three proteins, C, D and E.

![](image_url)

**Experiment 2.**
A 15 µl sample of Peak 1 from experiment 1 (proteins A&B) was loaded onto a 1.5 % agarose gel, made up in Tris barbitol buffer, pH 8.8. The sample was subjected to electrophoresis at 60 V for 45 min and the gel was then stained with Coomassie blue for 40 min. Destaining followed until the background was clear. The resultant destained gel is shown below.
**Experiment 3.**

Another aliquot of the protein mixture (A-E) was loaded onto DEAE cellulose at pH 5.0 using the same procedure as in experiment 1. The first peak eluted contained three proteins A, B and D. The second peak, C and E, was eluted after increasing the ionic strength of the buffer.

**Experiment 4.**

Another aliquot of the protein mixture (A-E) was loaded onto a G-50 Sephadex column (cut-off >50 000) equilibrated with 50 mM Tris pH 7.5. One mL fractions were collected after measuring a 30 ml void volume. Two protein peaks were eluted. The first peak contained one protein, A.

**Experiment 5.**

The original protein mixture was loaded onto an SDS-PAGE at pH 8.8. Three protein bands were observed after Coomassie blue staining: 15 000, 30 000 and 45 000.
Five (5) questions will be asked directly from this material

You wish to investigate the induction of \(\beta\)-galactosidase in a number of strains of \textit{E.coli}. You have been given the following protocol from a researcher’s lab notebook:

A wild type colony of \textit{E. coli} was selected and cultured in a medium which did not contain either glucose or lactose as carbon source. After sufficient growth was achieved IPTG was added to the culture to a final concentration of 0.5 mM. This time was denoted as time zero. At 2, 4, 8, 16 and 32 min after the addition of IPTG, 2 mL aliquots of culture were taken and assayed for \(\beta\)-galactosidase activity. The conditions for the assay were as follows:

Samples of culture (up to 50 % of the final volume) were incubated at 28\(^\circ\)C in a buffer containing 50 mM phosphate pH 7, 10 mM KCl, 5 mM MgSO\(_4\) and 20 mM \(\beta\)-mercaptoethanol. Substrate, \(o\)-Nitrophenol galactose (\(o\)-NPG) was added to a final concentration of 2.5 mM. The rate of the reaction was measured over a 10 min time period by monitoring the rate of appearance of the product, \(o\)-nitrophenol (\(o\)-NP), spectrophotometrically at 420 nm.

![Graph showing specific activity vs induction time](image-url)
Sample Questions

The table below shows 4 proteins (A – D) isolated from nuclear extracts from a cultured cell line. These proteins have nothing to do with the proteins described in the previous section.

<table>
<thead>
<tr>
<th>Protein</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular weight</td>
<td>100,000</td>
<td>14,000</td>
<td>12,700</td>
<td>64,000</td>
<td>68,000</td>
</tr>
<tr>
<td># subunits</td>
<td>2 equal</td>
<td>1</td>
<td>1</td>
<td>4 equal</td>
<td>1</td>
</tr>
<tr>
<td>pI</td>
<td>10.9</td>
<td>5.4</td>
<td>12</td>
<td>6.3</td>
<td>4.8</td>
</tr>
</tbody>
</table>

1. Which protein (A – E) could be a histone?

2. Which protein has the highest ratio of acidic side chains: basic side chains?

3. Using the column and buffers described in experiment 1 (DEAE cellulose, pH 7) proteins from the table above would elute BEFORE the high salt wash (0.5 m NaCl)?
   - A. Proteins A, D & E
   - B. Proteins B, D & E
   - C. Proteins B & C
   - D. Proteins A & C
   - E. None of the proteins

4. Which protein(s) from the table above would elute FIRST if they were loaded onto a Sephadex column as described in experiment 4?
   - A. Proteins A, D & E
   - B. Proteins B, D & E
   - C. Proteins A & C
   - D. Protein E
   - E. Proteins B & C

5. Which of the following statements concerning telomerases is INCORRECT?

Telomerase….  
   - A. catalyses the cleavage of the telomeres from chromosomes
   - B. is a type of reverse transcriptase
   - C. contains RNA
   - D. binds to a short repeat sequence at the 3’ end of the chromosome
   - E. activity is high in immortal cell lines
6. The Ames test relies on a number of factors. Which of the following is **NOT** an assumption required for the Ames test?

A. *Salmonella* mutate at a very fast rate  
B. Wild type *Salmonella* can synthesise histidine *de novo* (from scratch)  
C. Mutagenic compounds will only induce mutations which cause *His* reversions  
D. *Salmonella* deficient in histidine synthesis (*His--*) can be maintained on a medium with minimal histidine without proliferation  
E. A visible colony on the agar plate is the result of proliferation from a single cell

7. Photolyase results in light activated

A. Lysis of bacterial cell walls  
B. Cleavage of cyclobutyl bonds between pyrimidines  
C. Destruction of bacteriophage DNA  
D. Formation of a phosphodiester bond in the gap in the DNA backbone after repair  
E. Cleavage of purine bases from the sugar phosphate backbone

8. You are investigating an inheritable neurodegenerative genetic disorder caused by TNR (trinucleotide repeat) expansions. To have an effect in an individual:

A. The TNR expansion must ONLY occur in the genome of cells from the affected neuronal tissue  
B. The TNR expansion must be inserted into the coding region of the gene  
C. The TNR expansion must have occurred in the somatic cells and NOT the germline cells of the individual  
D. The TNR expansion cannot occur in the 5’ or 3’ UTR of the gene  
E. The gene containing the TNR expansion does not have to be expressed by the affected tissue

9. Genetic anticipation refers to:

A. The process of predicting the phenotype of an organism based on its genetic pedigree  
B. The increased severity of a mutation with each generation  
C. The prediction of a gene sequence based on its protein sequence  
D. The initiation of the second round of replication before the first round is completed  
E. The prediction of protein function based on gene sequence
10. *E. coli* has $4.6 \times 10^6$ base pairs in its genome. If the average bacterial protein has a molecular weight of ~40 000 and an average intergenic region of 500 bp what is the estimated number of different protein sequences coded for by the *E. coli* genome?

A. 100  
B. 1 000  
C. 3 000  
D. 5 000  
E. 10 000

11. Which of the following statements about the human genome is **INCORRECT**?

The human genome contains…

A. ~40% repetitive sequences  
B. multiple copies of ribosomal genes  
C. only 1 – 2% protein coding sequences  
D. introns which make up ~90% of most genes  
E. pseudogenes which are highly repetitive sequences

12. Which of the following is a **UNIQUE** feature of glucocorticoid up-regulation of PEPCK activity in liver during starvation?

A. Thiazolidinediones (TZDs) are artificial ligands for the glucocorticoid receptor  
B. Binding of the hormone results in the activation of adenyl cyclase  
C. Binding of the hormone to an intracellular receptor unmasks a nuclear localisation signal  
D. Stabilisation of the PEPCK mRNA in the cytoplasm  
E. Activation and migration of protein kinase A to the nucleus where it phosphorylates a transcription factor.
**Answers:**

1. C (small protein with high pI, +vely charged at pH 7)
2. E; the protein with the lowest pI will have the greatest # acidic:basic residues.
3. D; those proteins with pIs above 7 will be +ve at pH 7 and therefore repelled by the positive DEAE column
4. A; those proteins (A, D & E) with molecular weights >50,000 will be excluded from the gel filtration beads and hence elute faster.
5. A; Telomerase actually does the opposite it fills in and extends the telomeres using the RNA contained in the enzyme as a template and its reverse transcriptase activity. Its activity is high in immortal cell lines, preventing erosion of the telomeres with the rapid rate of cell division characteristic of these cell lines. If telomerase wasn’t active then apoptosis would occur once there was a critical loss of genetic material and the cells would rapidly die off.
6. C. The Ames test relies on a mutant strain of Salmonella which is deficient in Histidine biosynthesis de novo. This strain rapidly mutates anyway but if the compound tested is mutagenic will cause a higher frequency of ALL types of mutations, some of which will result in reversion. Those mutations will be able to proliferate on the minimal His medium forming colonies.
7. B; photolyase cleaves the cyclobutyl covalent bonds which form between adjacent pyrimidines on the same DNA strand.
8. E; remember Fragile X
9. B
10. C; proteins 40,000 mol. Wt. \(\rightarrow\) 400 amino acids (mol. Wt amino acid residue = ~100) \(\rightarrow\) 1200 bases + 500 intergenic = 1700. \(4.6 \times 10^5/1700 = 2,705 \rightarrow ~3000\). 
11. E is incorrect. Pseudogenes are not highly repetitive.
12. C is the unique feature of glucocorticoid activation. Glucocorticoids are steroid hormones, which are able to enter the cell, binding to an intracellular receptor which exposes a NLS. This enables the receptor hormone complex to enter the nucleus where it dimerises and binds to the glucocorticoid respsone element and hence activates the transcription of the PEPCK. TZDs work on PPAR\(\gamma\) not glucocorticoids making A wrong, glucagon binds and activates cAMP making B wrong, PEPCK mRNA is stabilised by both cAMP and cortisol so this feature is not unique to glucocorticoids thus D is wrong, and the activation of protein kinase A is brought about by increased cAMP not glucocorticoids. This question covers a lot of ground.