2015

BIOCHEMISTRY

Paper – BCT: 306

(Proteomics)

Full Marks – 25

The figures in the margin indicate full marks

Candidates are required to give their answers in their own words as far as practicable

Group – A

1. (i) Define the following terms which are used in proteomics

(a) MS spectra

(b) MS/MS spectra

(c) Protein identification.

(ii) What are the three essential parts of Mass Spectrometer ? Mention their function.

(iii) What is ICAT ? How is it possible to quantify protein and identify protein via ICAT strategy ? $(1\frac{1}{2} \times 3)+3\frac{1}{2}+(2+2\frac{1}{2})$

Or

2. (i) What are the limitations of gel based proteomics ?

(ii) What is DIGE ?

(iii) Discuss briefly the quantitation strategy and protein identification system using DIGE. Why is it necessary to introduce internal standard in DIGE system ?

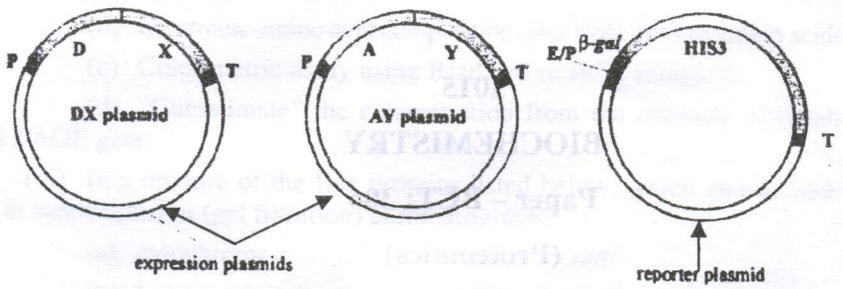
(iv) Differentiate between : ESI and MALDI.

(v) What precautions are taken in preparing protein samples for proteomics study ?

(vi) What is meant by Top Down and Bottom up approach in proteomic study ? $2+1+3+2+2\frac{1}{2}+2$

Group – B

3. Two mammalian proteins, designated X and Y, are studied in the following fictitious experiment using the yeast two-hybrid system. Expression plasmids functioning in yeast cells were used in the experiment (see figure).



They contained a promoter and a terminator region specific for yeast cells. One plasmid (designated DX plasmid) contained a fusion gene consisting of a cDNA coding for the DNA binding domain of GAL4 and a cDNA coding for protein X. The other plasmid (AY plasmid) contained a hybrid gene constructed from cDNA sequences coding for the activation domain of GAL4 and protein Y. A third, so-called reporter plasmid was also used in the experiment; it contained the HIS3 gene as a reporter gene hooked to the enhancer/ promoter region of the β-galactosidase gene. The three plasmids were transfected into a culture of mutant yeast cells that contained neither GAL4 nor HIS3 (GAL4, HIS3 mutant). The cells were spread on culture dishes with solid medium lacking or containing histidine as indicated in Table I. Study the experimental strategy, evaluate the results in Table I, and solve the following question :

The following statements are related to the information present in the description of the experiment. Based on the information given, select:

A. if the statement is supported by the information given;

B. if the statement is contradicted by the information given;

C. if the statement is neither supported nor contradicted by the information given.

Answer ***any five*** questions

(a) ___ The *HIS*3 gene of the reporter plasmid is constitutively expressed in the transfected mutant yeast cells. 1

(b) ___ The half-life of DX protein is shorter than that of GAL4. 1

(c) ___ X and Y proteins act as transcription factors in their mammalian host cells. 1

(d) ___ The _ -galactosidase enhancer does not function in the HIS3_ mutant cells. 1

(e) ___ The DX and AY fusion proteins can substitute the missing GAL4 activity in the transfected cells. 1

(f) ___ The DX fusion protein is able to bind to the β-galactosidase enhancer. 1

(g) ___ The AY fusion protein is able to activate RNA polymerase II. 1

4. Answer ***any five*** questions : 5×1

(i) For an application where you require a sample of your target protein at high purity, what would be a good purification strategy ? Assume that

your starting point is *E. coli* cells in which the target protein fused to an affinity tag has been over-expressed.

(a) Affinity chromatography followed by size exclusion chromatography

(b) Affinity chromatography only

(c) Affinity chromatography followed by ion-exchange followed by size exclusion chromatography

(d) Affinity chromatography followed by ion-exchange, followed by hydrophobic interaction and then size exclusion chromatography.

(ii) You know that the protein you want to purify from a natural source forms a multimer with multiple sub-units giving a molecular weight in solution much bigger than visualised denatured on SDS-PAGE. There is only a small amount of the target protein in the total protein sample. Which of the following is an appropriate purification strategy ?

(a) Size Exclusion Chromatography

(b) Ion Exchange Chromatography followed by Size Exclusion Chromatography

(c) Ion Exchange Chromatography

(d) Affinity Chromatography followed by Size Exclusion Chromatography.

(iii) You find that your protein sample loses activity during storage. What can you do about this ?

(a) Add an additional purification step

(b) Use a protease inhibitor during purification steps

(c) Perform each step as quickly as possible, in a cold-room.

(iv) Which of these techniques is often considered a suitable "polishing" step in a protein purification strategy ?

(a) Affinity chromatography

(b) Ion-exchange chromatography

(c) Hydrophobic interaction chromatography

(d) Size-exclusion chromatography.

(v) Your purification strategy of combinations of chromatography steps gives a protein preparation with a single band on SDS-PAGE. Which of the following would be best for determining the protein concentration (as mg/ml or molarity) ?

(a) Measure a UV absorbance scan and use the absorbance at 280nm with the molar extinction coefficient (predicted from the amino acid sequence)

(b) Determine amino acid composition after hydrolysis to amino acids

(c) Colorimetric assay using Bradford or BCA assays

(d) "Guesstimate" the concentration from the intensity of bands on SDS-PAGE gels.

(vi) In a mixture of the five proteins listed below, which should elute second in size-exclusion (gel filtration) chromatography ?

(a) cytochrome c MW = 13,000

(b) immunoglobulin G MW = 145,000

(c) ribonuclease A MW= 13,700

(d) RNA polymerase MW = 450,000

(e) serum albumin MW = 68,500.

(vii) The ionic strength of 1 N $(NH_4)_2SO_4$ is equivalent to

(a) 1 (b) 2 (c) 3 (d) 4

5. Answer ***any five*** questions : ½ × 5

(i) The ultrasonic vibrations have frequency of

(a) Below 20 Hz (b) Above 20 KHz

(c) Equivalent to 10,000 Hz (d) Above 50 MHz

(ii) The most effective anion in salting out is

(a) SO_4^-. (b) PO_4^- (c) Cl^- (d) NO_3^-

(iii) Denaturation of proteins leads to loss of biological activity by

(a) Formation of amino acid

(b) Loss of primary structure

(c) Loss of both primary and secondary structure

(d) Loss of secondary and tertiary structure.

(iv) At isoelectric point, a protein has its

(a) Maximal solubility (b) Minimum solubility

(c) Maximum charge (d) Minimum activity.

(v) The purity of an enzyme at various stages of purification is best measured by :

(a) Total protein (b) Total enzyme activity

(c) Specific activity of the enzyme (d) Percent recovery of protein.

(vi) Protein of pI value 5.8 in running buffer of pH 8.3 will move towards

(a) Anode (b) Cathode (c) Any direction (d) Won't move.

(vii) Dialysis involves the movement of a

(a) charged solute molecule (ion) across a membrane.

(b) gas molecule across a membrane.

(c) nonpolar solute molecule across a membrane.

(d) polar solute molecule across a membrane.

(e) water molecule across a membrane.