

BIOSE10 - Lecture 3 Practice Problems - Answers

1-

- (a) Ion-exchange chromatography separates proteins on the basis of their charges. (b) Size-exclusion or gel filtration chromatography separates on the basis of size (c) Affinity chromatography separates proteins with specific, high affinity for some ligand (attached to an inert support) from other proteins with no such affinity.

2-

- (1) Use Edman degradation to determine the sequence of each peptide. (2) Create a second set of peptides by treatment of the protein with a specific protease (e.g., trypsin), and determine the sequence of each of these. (3) Place the peptides in order by their overlaps.

3-

From the percentage recovery of activity (units), we calculate percentage yield and specific activity (units/mg).

| Procedure | Protein (mg) | Activity (units) | % Yield | Specific activity (units/mg) | Purification factor (overall) |
|-----------|--------------|------------------|---------|------------------------------|-------------------------------|
| 1 | 20,000 | 4,000,000 | 100 | 200 | 1.00 |
| 2 | 5,000 | 3,000,000 | 75 | 600 | × 3.0 |
| 3 | 4,000 | 1,000,000 | 25 | 250 | × 1.25 |
| 4 | 200 | 800,000 | 20 | 4,000 | × 20 |
| 5 | 50 | 750,000 | 19 | 15,000 | × 75 |
| 6 | 45 | 675,000 | 17 | 15,000 | × 75 |

(b) Step 4, ion-exchange chromatography; this gives the greatest increase in specific activity (an index of purity and degree of increase in purification).

(c) Step 3, pH precipitation; two-thirds of the total activity from the previous step was lost here.

(d) Yes. The specific activity did not increase further after step 5. SDS polyacrylamide gel electrophoresis is an excellent, standard way of checking homogeneity and purity.

4- HSEGTFSNDYSKYLEDRKAQDFVRWLMNINKRSGAAE

19 3 Practice Problems Answers

G Orfield



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