

Biochemistry for dental students Practice

Question 1:

You have a pentapeptide that is neutrally charged <u>at physiological pH</u>. Based on the observations below, determine the sequence of the peptide knowing that it is composed of gly, lys, glu, phe, and met (not in order).

Experiment 1: cleavage of the pentapeptide with trypsin generates a tripeptide and a dipeptide. The dipeptide is positively charged and the tripeptide is negatively charged.

Experiment 2: cleavage of the pentapeptide with cyanogen bromide generates a single amino acid and a tetrapeptide that is neutrally charged.

Experiment 3: cleavage of the pentapeptide with chymotrypsin generates a dipeptide that is negatively charged and a tripeptide that is positively charged.

Experiment 4: cleavage of the pentapeptide with elastase results in no cleavage.

Experiment 5: cleavage of the pentapeptide with pepsin generates a negatively charged tripeptide and a positively charged dipeptide.

Question 2:

You have performed chromatographic techniques on 4 proteins (W, X, Y, and Z). Based on the experiments below, answer the questions.

Technique	Order of elution (after washing out unbound proteins)
Gel-filtration chromatography	Y, W, Z, then X
Cationic-exchange chromatography	Z then Y
Anionic exchange chromatography	W then X

- A. Which protein has the highest pl?
- B. Which protein has the lowest pl?
- C. If SDS-PAGE is performed, what is the order of proteins (from top to bottom)?
- D. If two-dimensional SDS-PAGE is performed, which protein will be located at the leftmost bottom of the gel?
- E. You performed an immunoblot for a mix of the four proteins. You got a single band representing Y. Can you use the same antibody in affinity chromatography?



Solutions

Question 1

Note: you need to know the amino acid abbreviations and their properties.

Note: you need to know the enzymes and chemicals, their recognized amino acids, and their cleavage sites

Hint: all peptides will have an amino group and a carboxyl group.

Experiment 1: Cleavage occurs at a lysine. The dipeptide contains lys and the tripeptide contains glu. The amino acid order of the peptide is (1-lys-3 -4-5).

Experiment 2: The single amino acid is met and it is at the N-terminus. The tetrapeptide contains all other four amino acids. The order of the amino acids is (met-lys-3-4-5).

Experiment 3: Cleavage occurs at the C-terminus of phe. The dipeptide contains glu and the tripeptide contains lys and phe. The order of the amino acids is (met-lys-phe-4-5)

Experiment 4: Gly is at the C-terminus. The order of the amino acids is (met-lys-phe-4-gly). You should know by now what amino acid 4 is. Anyhow, go on.

Experiment 5: Cleavage occurs at the N-terminus of phe. The order of the amino acids is (met-lys-phe-glu-gly)

Question 2:

- A. Y, since it is the one eluted last with the cationic-exchange chromatography.
- B. X, since it is the one eluted last with the anionic-exchange chromatography
- C. Top has highest molecular weight and vice versa. The order in the SDS-PAGE is Y, W, Z, and finally X.
- D. Leftmost bottom: a smaller MW with lowest pl. It is X.
- E. Yes, because the antibody is specific for protein Y.