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Biochemistry for dental students
Practice

Question 1:
You have a pentapeptide that is neutrally charged at physiological pH. 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 pI?
B. Which protein has the lowest pI?
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 pI. It is X.
E. Yes, because the antibody is specific for protein Y.