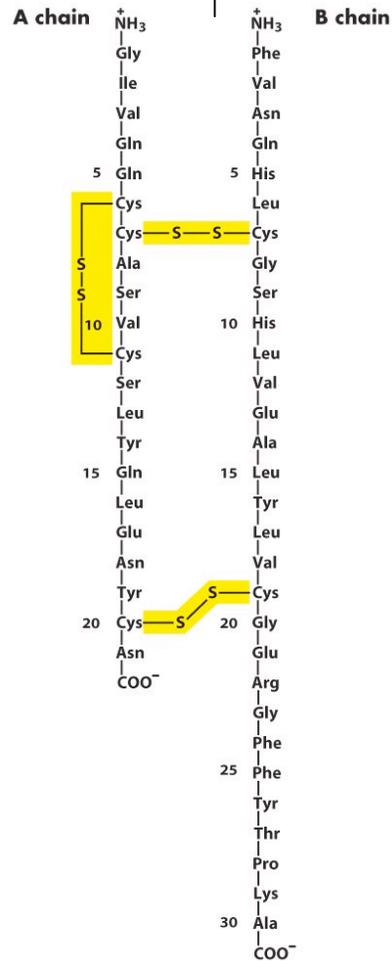


Worksheet #1

#1. The protein pictured below is bovine insulin. Determine the number of fragments and the size of each fragments that would be generated upon treatment with the following:

	Without DTT	With DTT
Trypsin		
Chymotrypsin		
BrCN		



<u>Reagent (source)</u>	<u>Specificity</u>
Trypsin (bovine pancreas)	Lys, Arg (C)
Chymotrypsin (bovine pancreas)	Phe, Trp, Tyr (C)
Staphylococcus V8 protease	Glu, Asp (C)
Pepsin (porcine pancreas)	Phe, Trp, Tyr (N)
Cyanogen bromide (chemical)(CnBr)	Met (C)

#2. A peptide was cleaved into smaller fragments using chymotrypsin and CnBr. The fragments were separated by HPLC and then sequenced by Edman degradation. Determine the sequence of the original peptide from the following fragment sequences.

Cleavage with chymotrypsin

EAGPDGMECAF
GPF
EAAMCKW
HR
IAHTY

Cleavage with CnBr

ECAFHR
CKWEAGPDGM
IAHTYGPFEAAM

#3. The following three peptides are subjected to anion-exchange chromatography at pH 7.5 using a NaCl gradient to elute the peptides. There are no disulfide bridges. What is the charge of each peptide at pH 7.5 and in what order will they elute from the column? Use Table 3.2 to find the pKa values.

(1) MARKER (2) SADDLE (3) CRACKED

#4. A small peptide was found to contain equimolar amounts of the following amino acids: arginine, glutamic acid, glycine, lysine, methionine and phenylalanine. Individual samples of the peptide were treated with the following agents with the results noted:

- a) trypsin: arginine and a pentapeptide
- b) cyanogen bromide: two tripeptides
- c) *S. aureus* V8: lysine and a pentapeptide
- d) chymotrypsin: a dipeptide and a tetrapeptide, with the latter showing absorbance at 260nm.

What is the primary structure of the peptide? Explain each piece of evidence given and the reasoning that led to your answer.

#5 You have 2 globular proteins. Protein X has 300 amino acids and protein Y has 400 amino acids. Which one will emerge first using gel filtration chromatography?

#6 You have 2 globular proteins. Protein X has 300 amino acids and protein Y has 400 amino acids. Which one will emerge first using gel filtration chromatography?

#7 Answer True or False

- a) Reverse-phase chromatography (HPLC) separates peptides based on molecular weight.
- b) One method used to prevent disulfide bond interference with protein sequencing procedures is to reduce disulfide bridges and prevent their reformation with the addition of iodoacetic acid.
- c) High molecular weight proteins will migrate farther during gel electrophoresis (SDS-PAGE).
- d) β -sheet protein structures can be stabilized by hydrogen bonding between distant residues on the same polypeptide.
- e) β -sheets are a type of secondary structure and are found in every protein.
- f) In the α -helix, the hydrogen bonds that stabilize the helix occur mainly between electronegative atoms of the R groups.
- g) Negatively charged peptides flow through a cation-exchange chromatography column without binding.
- h) The enzyme hexokinase (972 amino acids) will elute from a gel filtration column before myoglobin (155 amino acids).
- i) Separation of proteins in the first dimension of 2D gel electrophoresis is based on a protein's molecular weight.

