

Biochemistry questions assignment



**ASSIGN
BUSTER**

The column is washed with a pH 7.2 HEPES buffer (50 mM) and the peptide does not elute. Suggest two ways that you can change the buffer that will make the peptide elute from the column. 2) What amino acid(s) is/are involved in crosslink polypeptide chains. How can these crosslink be cleaved and prevented from reforming? 3) A polypeptide is subjected to the following degradation techniques results in polypeptide fragments with the indicated amino acid sequences.

What is the amino acid sequence of the entire polypeptide? A) Trying digestion: 1) Glen-Met-Lays 2) Ugly-Met-Asp-ell-Lays 3) Phew-Ala-Met-Lays 4) Try-Rag B) Cyanogens bromide treatment: 5) Asp-Ill-Lays-Glen-Met 6) Lays 7) Lays-Phew-Ala-Met 8) Try-Rag-Ugly-Met 4) While on an expedition to the Amazon Jungle, you isolate a polypeptide you suspect of being the growth hormone of a newly discovered species of giant spider. Unfortunately, your portable sequencer was so roughly handled at the airport that it refuses to provide the sequence of more than four consecutive amino acid residues.

Nevertheless, you persevere and obtained the following data: Hydrolysis's: Vial Dandy's chloride treatment followed by acid hydrolysis: Dandy's-Pro Trying digestion followed by Demand degradation of the separated fragments: Ugly-Lays Phew-ell-Vial Pro-Ugly-Ala-Rag Seer-Rag A) Provide as much information as you can concerning the amino acid sequence of the polypeptide. B) Considering the poor condition of your sequencer, what additional analytical technique would most conveniently permit you to complete the sequence determination of the polypeptide?

C) Suggest two ways that you would use to separate the tropic peptides mentioned above if you needed large quantities of each peptide. If you needed only analytical quantities of the peptides. 5) A hepatitis when treated with trypsin produced two peptides: TTL (Asp, Ugly, Try) and TO (Ala, Lays, Phew, Vial). When the hepatitis was treated with chymotrypsin, three peptides were produced; CT 1 (Ugly, Lays, Try), ACT (Ala, Phew, Vial) and ACT (Asp). (The parentheses indicate an unknown sequence.) When the original peptide was treated with 2, 4-dinitrofluorobenzene and hydrolysis, DEN-Lays and DEN- Ala were recovered.

What is the amino acid sequence of the hepatitis? What is the structure of DEN-Lays? 6) Three chymotrypsin fragments in the problem above were chromatographic on an anion exchange column at pH 7. 9. (Resin is positively charged.) What elution pattern would you expect if: A) you used UV absorption at 280 nm to detect the peptides B) you use UV absorption at 220 nm to detect the peptides C) you treated the effluent with unhidden and the absorbency of the unhidden adduct is measured. 7) Predict the secondary structure in which the following sequence is found. Be sure to clearly indicate your reasoning. Lee-Seer-Glyc-Met-Thro-Sys-Lys-Rag-Ala-Ala-Vial-Glyc-Assn-Lys-Lays- 8) Describe the hierarchy of forces important for formation of tertiary structure in proteins. Predict the forces (bonds) perturbed by a) extremes in pH, b) organic solvents and c) detergent molecules. How do these reagents perturb each interaction? 9) Differentiate among a domain, motif, secondary structure, and tertiary structure. 10) Studies of naturally occurring amino acid substitutions in protein (mutations) have revealed the following: -Replacement of Seer is least likely to eliminate

protein function. Replacement of Trip is most likely to eliminate protein function. -Changes such as Lays to Rag and Ill to Lee often have little effect on protein function. -Ugly residues are often conserved at certain positions within protein chains. Using your knowledge of properties of amino acids and of protein structure/folding, give a rationale for each of the above observations. 1 1) Explain how it is that in SD gel electrophoresis the largest proteins move lowest while in gel exclusion chromatography largest proteins move through the column fastest.