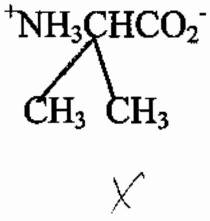
BCH210 Term Test 1.
October 12, 2010, 6:00-8:00 PM.
Answer <u>all</u> questions (1 mark each).

1. Consider the structures of the four amino acids shown below:



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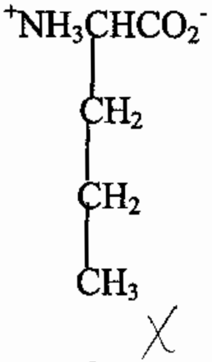

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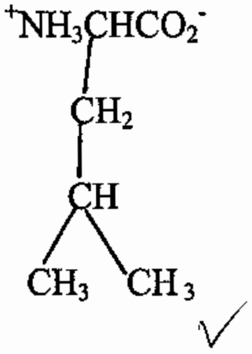

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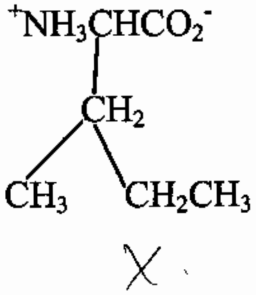


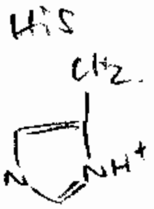
4

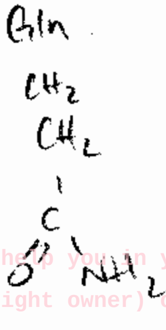
Among the side chains in the above structures, which correspond to those found in the 20 commonly-occurring amino acids?

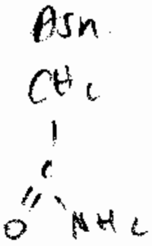
(a) 1 and 4
(b) 2 only
(c) 3 and 4
[✓ (d) 3 only]

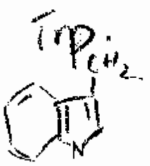
2. Consider the group **His, Gln, Asn, Trp, Lys,** and **Arg** among the 20 commonly-occurring amino acids. What property is characteristic of their side chains?

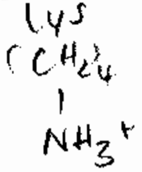
(a) They are the complete group of amino acids that has at least one nitrogen atom in their side chains.
(b) They represent all except two of the amino acids that have at least one nitrogen atom in their side chains.
(c) They represent the complete group of amino acids that do not contain at least one methyl group in their side chains.
(d) They represent the complete group of amino acids that have polar side chains.

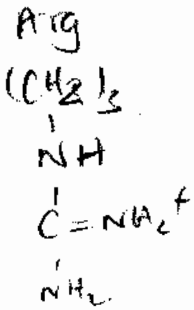












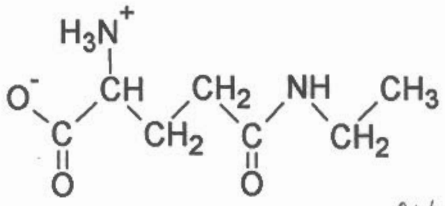
3. The overall (net) charge of a 40-residue peptide containing two each of the 20 commonly-occurring amino acid at physiological pH (7.4) is:

(a) +2
[✓ (b)] 0
(c) +4
(d) - 2

4. The six-residue peptide **Asp-Asp-Asn-Arg-Arg-Gln** is dissolved in a water solution at pH 8.5. When the pH is lowered to 2.0, what is the change in the net charge of the peptide? (Ignore the charge of the N- and C-terminal backbone groups.)

(a) From +2 to 0.
[✓ (b)] From 0 to +2.
(c) From -3 to 0.
(d) There is no net change in charge.

5. Consider the structure of the amino acid shown below. It is not one of the 20 commonly-occurring amino acids, but is derived from one of them. Which amino acid is the structure derived from?



CH2
CH2
C=O
NH
CH2
CH3

CH2
CH2
C
O NH2

(a) Asparagine
(b) Aspartic acid
[✓ (c)] Glutamine
(d) Arginine

6. Suppose you added two droplets of water into a beaker filled with the hydrocarbon liquid hexane. In principle, the "hydrophobic effect" would predict that:

(a) Each water droplet will split into half its size, and become completely surrounded by hexane molecules.
[✓ (b)] The two water droplets will coalesce to form one larger water droplet. ✓
(c) Each water droplet will split into half its size, and then break up into thousands of smaller droplets, each surrounded by hexane.
(d) The hexane will interact directly with the water, creating an essentially infinite chain of alternating hexane and water molecules.

1 2 3 4 5

7. A largely α-helical peptide has the sequence **Ile-Tyr-Thr-Met-Lys-Trp-Gln-Ile-Glu-Gly-Phe-Ser-Ala** (reading from the N-terminus to the C-terminus). In such a helix, the carbonyl oxygen of the Tyr residue is H-bonded to a side chain N-H proton of:

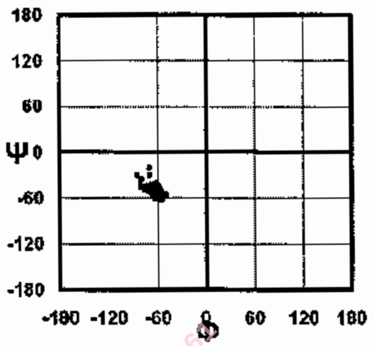
(a) Lys
[✓ (b)] Trp
(c) Gln
(d) None of the above

8. Ramachandran plots made from (ϕ,ψ) angles of individual amino acids [such as Ala or Arg] - compiled from structures of crystallized proteins - consistently show that essentially <u>all points occur</u> in the α-helix or β-sheet regions. Yet these proteins are known to have significant regions of "unstructured" (random) structure. <u>Excluding</u> the special cases of Gly and Pro, what is the <u>most probable</u> explanation for this observation?

X (a) "Unstructured" regions are never included in Ramachandran plots.
? [✓ (b)] Individual residues in "unstructured" regions likely contain some combination of (ϕ,ψ) angles which also have α-helical or β-sheet values.
X (c) The (ϕ,ψ) values of residues in "unstructured" regions are the mirror images of the usual α-helix and β-sheet (ϕ,ψ) values.
X (d) Ramachandran neglected to include "unstructured" regions in designing his plot.

9. Assume that it is possible to obtain a Ramachandran plot not only for globular proteins but also fibrous proteins. ϕ = phi; ψ = psi. You are given that the α-helix (phi, psi) angles are centered at (-60°, -50°); the β-sheet (phi, psi) angles are at (-140°, +135°); and the poly-proline helix (phi, psi) angles are at (-60°, +135°). Which set of descriptions matches MOST closely to the following four Ramachandran plots?

1



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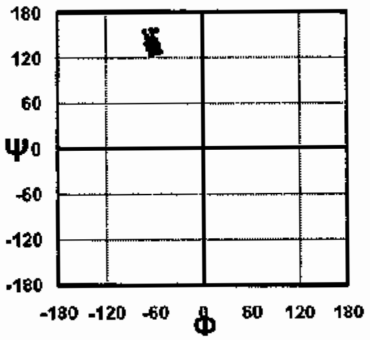

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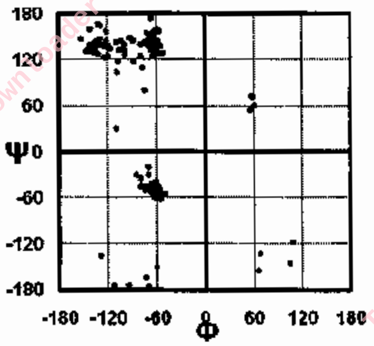

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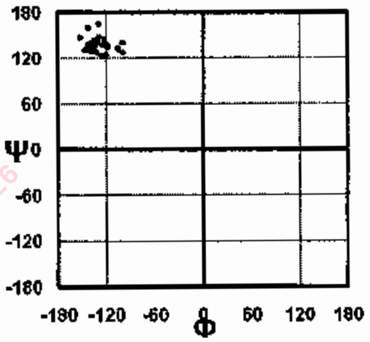


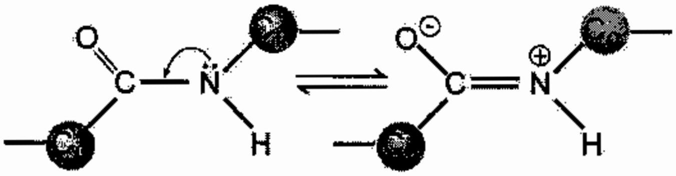
(a) 1- a segment of keratin that may contain a HIGH amount of cysteine residues; 2 - a segment of keratin that may contain a LOW amount of ✗ cysteine residues; 3 - a typical globular protein; ✓ 4 - a segment of silk. ✓

✗ (b) 1 - a typical globular protein; 2- a segment of silk; 3 - a segment of keratin; 4 - a segment of collagen. ✗

[✓ (c)] 1 - a segment of keratin; ✓ 2 - a segment of collagen; ✓ 3 - a typical globular ✓ protein; 4 - a segment of silk. ✓

(d) 1- a segment of keratin that may contain a LOW amount of cysteine residues;
✗ ✓ 2 - a segment of collagen; 3 - a typical globular protein; ✓ 4 - a segment of keratin that may contain a HIGH amount of cysteine residues.

10. Consider the diagram shown below. Which of the following BEST describes a phenomenon illustrated in the diagram?



- (a) Rotation around the C-N portion of the peptide bond depends largely on the sizes of each of the Cα atoms. x
- (b) Rotation around the C-N portion of the peptide bond is restricted because the two Cα atoms repel each other. x
- (c) The C-N portion of the bond is a resonance hybrid of the two structures shown, and therefore rotation about this bond becomes much easier than a normal single bond. x
- [✓ (d)] Rotation around the C-N is restricted, causing the C, N, O, and H atoms to be held in a flat, planar arrangement. ✓

11. Gelatin differs from collagen in the following way:

- x (a) The polyproline helical structure of the individual strands of native collagen has been converted to α-helical strands.
- (b) The native strands of collagen have been thermally broken down into shorter peptides.
- (c) The hydroxyproline residues have formed a chain of side chain-side chain hydrogen bonds within each strand.
- [✓ (d)] The collagen triple helix has been denatured, and new hydrogen bonds have formed irregularly between various individual protein strands. ✓

12. Folding of globular proteins is believed to be "directed" because proteins fold to their native structures in orders-of-magnitude less time than they would if the protein chain folded randomly. Which one of the following events does <u>NOT</u> apply to the "directed" nature of protein folding?

- T (a) Interaction of short segments of α-helices in the freshly synthesized protein chain.
- T (b) Formation of tertiary structure promoted by interactions of short segments throughout the protein.
- [✓ (c)] Additional peptide bond formation immediately after biosynthesis of the full chain.
- T (d) Hydrogen bond formation between two short segments to form β-sheet structure.

13. Denatured reduced ribonuclease re-folds correctly once both β-mercaptoethanol <u>and</u> urea have been dialyzed out. However, the ribonuclease disulfide bonding pattern becomes "scrambled" if urea is left in during refolding. What is the most likely role of urea in these processes?

✓ [✓ (a)] Urea competes for hydrogen bonds in the native protein, such as those that stabilize α-helix and β-sheet secondary structure as well as β-turns.
X (b) Urea forms covalent bonds with -SH groups, thus preventing formation of the structure $-CH_2-S-S-CH_2-$.
X (c) Urea reacts with β-mercaptoethanol, thus preventing formation of $-CH_2-SH$ groups.
X (d) Urea competes for hydrogen bonds with those in the native protein, thus interrupting native covalent interactions.

14. Consider the properties of amino acids and the types of forces that dictate protein folding. Which of the following protein mutations would most likely NOT affect the normal folding of a typical globular protein?

NP P
(a) An Ile residue within the hydrophobic core mutated to a Gln residue. X
(b) An Asn residue whose side chain is involved in a hydrogen bond mutated to an Ala residue. X
[✓ (c)] A Ser residue on the surface of the protein mutated to the β-branched residue Thr. ✓
(d) At neutral pH, an Asp residue involved in a charge-charge interaction with an Arg residue mutated to a Lys residue.
X

15. Native folded structures of globular proteins do not denature spontaneously. This is true because:

(a) Entropy is lost during unfolding.
(b) Pairs of α-helices nucleate unfolded protein chains at an early stage.
(c) In the unfolded state, portions of the protein have greater entropy.
[✓ (d)] A large energy barrier must be surmounted to convert the protein to an almost-folded state.

Trypsn.
KR

Chymo.
FWY

16. Consider the peptide chain **ADYYAARRAAKKAALLEAMVM**. You treat the peptide chain with trypsin, then take these fragments and treat them with chymotrypsin, and lastly treat these chymotrypsin products with cyanogen bromide (CNBr). Considering these final products following the three treatments, how many fragments are produced that have one or more residues with charged side chains at pH 7?

(a) 3
(b) 4
(c) 5
[✓ (d) 6]

17. You are given a mixture in aqueous solution of equal amounts of three peptides having the known sequences i, ii, and iii as shown below, and wish to use various types of chromatography to separate this mixture.

(i) **HHHHHHHHHHHHHHHHHHH**
(ii) **ALALALALALALALALALALALALALALALALALALAL**
(iii) **ALALALALALALALALALAL**

Consider the order of retention times (shortest time to longest time) that you would <u>predict</u> that the three peptides should wash out (elute) from <u>each</u> of the following three columns: HPLC, gel filtration, and nickel affinity. Which ONE of the following statements BEST describes the expected behaviour of these three peptides on the three types of columns?

✓ [✓ (a)] The order of elution from the HPLC column would be first (i), then (ii) and (iii), although it may not be possible to predict the elution order of (ii) and (iii) since they have the same overall hydrophobicity.

✓ (b) The order of elution from the gel filtration column would be first (ii), then (i) and (iii), although it may not be possible to predict the elution order of (i) and (iii) since they have approximately the same size.

X (c) The order of elution from the nickel affinity column would be first (i), then (ii) and (iii), although it may not be possible to predict the elution order of (ii) and (iii) since neither of them will interact with the affinity column.

X (d) All three statements (a), (b), and (c) are correct.

no gel.

18. You have a mixture that contains 75% of protein A and 25% of protein B. The two proteins are exactly the same size, and have the same amino acid composition, but differ significantly in amino acid sequence. One of the proteins has a more hydrophobic surface than the other, but you don't know which. What laboratory techniques would be the BEST FIRST ATTEMPT toward separating A and B and determining their amino acid sequences?

(a) Affinity chromatography followed by treatment with carboxypeptidase.
(b) Gel chromatography followed by treatment with trypsin and chymotrypsin.
[✓ (c)] HPLC followed by a combination of Edman degradation and carboxypeptidase treatment.
(d) HPLC followed by ion exchange chromatography.

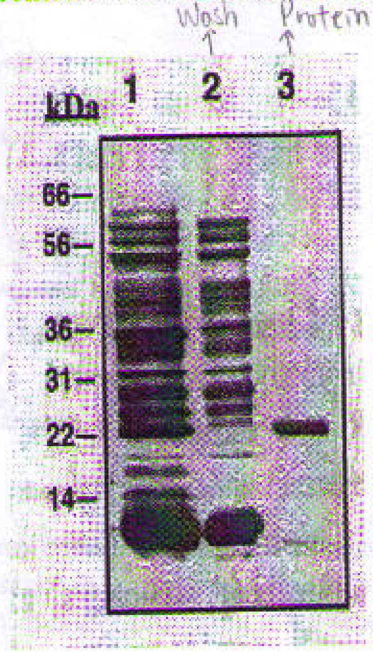
19. You are asked to gather some information about the sequence of a 10-residue peptide that is highly fluorescent. You perform the Edman degradation on the peptide 3 times, and each time you get the same amino acid. After a total of 5 Edman cycles, you find that the remaining 5-residue peptide is still fluorescent. You then find that treatment of the original 10-residue peptide with cyanogen bromide has no effect. Which ONE of the following sequences <u>cannot</u> be a <u>possible</u> primary sequence for this peptide?

(a) WWWWWWWWWW
(b) WWWWWWWWWM
[✓ (c)] NNNWWNNNNM ✗
(d) WWWNNNNNWW ✓

20. You wish to use *E.coli* bacteria to express a mutant insulin, which is a protein that the bacteria do not naturally contain. You have a double-stranded DNA plasmid that contains two palindromic sites for the restriction endonuclease from *S. achromogenis*. What is the correct order of experiments for performing this task?

(a) Treat the plasmid with the restriction enzyme, treat the plasmid with DNA ligase, prepare a synthetic oligonucleotide coding for the desired insulin sequence, add the restriction enzyme back to the plasmid, insert the plasmid into the *E. coli*, and add DNA polymerase.
(b) Prepare a synthetic oligonucleotide coding for the desired insulin sequence, treat the oligonucleotide with DNA polymerase, add the restriction enzyme to the plasmid, insert the plasmid into the *E. coli*, and treat with DNA ligase.
(c) Transform the plasmid into *E. coli*, add the required restriction enzyme, add DNA ligase, and synthesize an oligonucleotide coding for the desired insulin sequence.
[✓ (d)] Prepare a synthetic oligonucleotide coding for the desired insulin sequence, treat the plasmid with the restriction enzyme, add the oligonucleotide to the plasmid, treat with DNA ligase, and transform the plasmid into the *E. coli*. ✓

✓21. Lane 1 in the diagram below shows an SDS-PAGE gel of *E. coli* proteins obtained from lysing the cell after expression of a 22,000 MW protein (shown at 22 in the diagram) that you have engineered into a plasmid. Your engineered protein also contains a sequence of six His residues covalently attached to its C-terminus. What statement below is most consistent with the results shown in lanes 2 and 3?



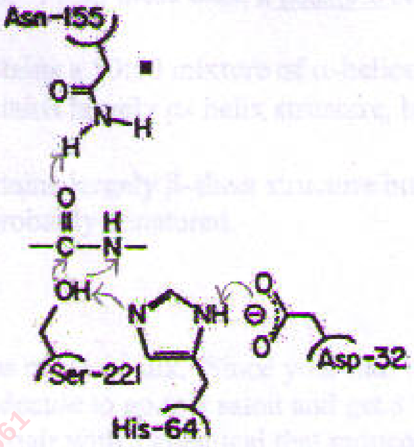
(a) Lane 2 shows the protein mixture before it has been poured down a nickel affinity chromatography column. Lane 3 shows the mixture after it has been poured down the nickel affinity column.

(b) Lane 2 shows the mixture before it has been poured down a nickel affinity chromatography column. Lane 3 shows the mixture after it has been poured a second time down the nickel affinity column.

(c) Lane 2 shows the mixture before it has been washed off the nickel affinity column with imidazole. Lane 3 shows the mixture after it has been washed off the nickel affinity column with urea.

[✓ (d)] Lane 2 shows the mixture after it has been poured down a nickel affinity chromatography column. Lane 3 shows the mixture after it has been washed off the nickel affinity column with imidazole.

✓ 22. The diagram below illustrates the charge relay network of the Asp, His, and Ser residues of a serine endopeptidase enzyme, shown with a susceptible peptide bond substrate and a neighboring Asn residue. Which of the following statements BEST describes the catalytic action of the enzyme?



[✓ (a)] If a hydrogen bond forms between the His-64 nitrogen atom (the one on the left in the diagram), this will assist in giving partial negative character to the Ser-221 side chain oxygen atom, thereby activating it for attack on the substrate peptide bond carbonyl group.

(b) The peptide bond of the substrate shown between the Asn-155 and the Ser-221 OH group has the effect of blocking the interaction between the Asn side chain and the Ser side chain.

(c) The Asp-32 contributes a positive charge to the charge relay network, allowing the NH on the His-64 imidazole ring to move to the other nitrogen atom.

(d) The Asn-155 side chain N-H proton inactivates the His-64 by forming a hydrogen bond with the N-H proton of the peptide substrate.

✓ 23. D C.

E D R K

Casette mutagenesis experiments established that mutation to Glu, Asp, Arg, or Lys at subtilisin position Met-222 essentially inactivated the enzyme. Which conclusion can you draw from these results?

(a) In natural subtilisin, the minimum steric volume of a side chain at position 222 required for at least some activity is equivalent to that of the Met side chain.

(b) Side chains of minimum steric volume are the least likely to impact on the environment of the neighboring active site Ser-221.

(c) In natural subtilisin, the Met-222 side chain is probably involved in a hydrogen bond via the S-atom to the side chain carboxamide C=O group of Asn-155.

[✓ (d)] Polar side chains probably disrupt the charge-relay system at the enzyme active site.

24. You are studying the secondary structure elements of a protein, and have performed infra-red (IR) spectroscopy and circular dichroism (CD) spectroscopy experiments on the protein dissolved in water at pH 7.0. You find that the IR spectrum contains a band at 1640 cm^{-1}, but no other bands in the Amide I region; and that the CD spectrum contains a major negative band near 200 nm but no other significant bands at higher wavelengths. From these data, a possible conclusion is:

(a) The protein contains a 50:50 mixture of α-helices and β-sheets.
(b) The protein contains largely α- helix structure, but becomes unstable during the experiment.
X (c) The protein contains largely β-sheet structure but little or no tryptophan.
[✓ (d)] The protein is probably denatured.

25. Suppose your hair was made of silk. Since your hair is straight and you would like to make it curly, you decide to go to a salon and get a "perm" treatment. The beautician treats your hair with a chemical that reduces disulfide bonds, and then exposes your hair to the air to quickly re-oxidize any disulfide bonds. What is the most likely outcome of this treatment?

[✓ (a)] Your hair has become curly.
X (b) Your hair has been temporarily transformed into keratin.
X (c) Your hair remains straight but will soon become curly as it grows.
(d) Your hair remains straight. So you just go home, wash your hair, and decide to have something else for dinner instead of cabbage and onions with lots of garlic.

-END-