BCH 4053 February 28, 2003

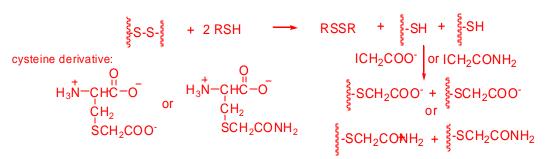
HOUR TEST 2

NAME____

KEY

Describe two methods of cleaving disulfide bonds prior to sequencing Page Points (10)1. a protein, indicating the reagents used in each case and the structure of the cysteine residue produced after the cleavage. 1 1 pt rxn, 2 pts reagent(s), 2 pts cysteine derivative structure 2 3 (a) Performic acid oxidation 4 Total 100

(b) Reduction followed by alkylation



(6) 2. A peptide was subjected to the following degradative techniques resulting in the fragments with the indicated sequences.

I. Cyanogen bromide treatment

Asp-Ile-Lys-Gln-Met Lys-Val-Ser Lys-Phe-Ala-Met Tyr-Arg-Gly-Met

II. Trypsin hydrolysis

Gln-Met-Lys Gly-Met-Asp-Ile-Lys Phe-Ala-Met-Lys Tyr-Arg Val-Ser

Give the complete sequence of the original peptide.

Tyr-Arg-Gly-Met-Asp-Ile-Lys-Gln-Met-Lys-Phe-Ala-Met-Lys-Val-Ser

6 pts complete sequence, 3 pts if all but one peptide aligned properly, 0 pts if more than one missed

(6) 3. Fill in the following blanks with **a helix** or **disordered** to represent the primary conformation of the indicated synthetic polypeptide at the indicated pH.

1 pt each	
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Polypeptide	pH 2	pH 7	рН 12
polyglutamate	<u>a helix</u>	<u>disordered</u>	<u>disordered</u>
polylysine	disordered	disordered	<u>a helix</u>

BCH 4053 Hour Test-2			Page 2		Name <u>KEY</u>	
(12)	4.	Given the followin	g data on five diff	erent proteins:		
		Protein	M.W	D	pI	
		cytochrome c	13,400	11.4	10.6	
		fibrinogen	330,000	2.0	5.6	
		serum albumin	65,000	5.9	4.8	
		ovalbumin	45,000	7.8	4.6	
		me all proteins have j		umes of about 0	.73 ml/g. Indicate in the blanks	the

protein(s) with the indicated behavior.

3 pts each blank _____serum albumin_

<u>cytochrome c</u> (b)

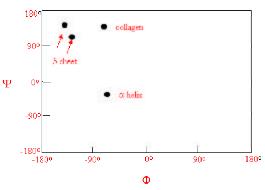
serum albumin and ovalbumin

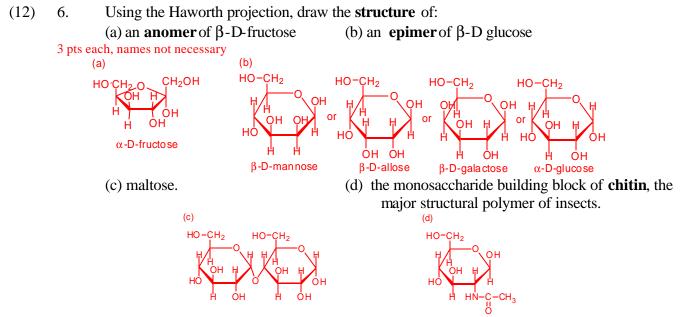
<u>cytochrome c</u> (d)

- Does not bind to a DEAE column at pH 7.4. (i.e., an anion exchange column).
- Does not bind to a carboxymethyl cellulose column at pH 5.0. (i.e., a cation exchange column).
- Migrates fastest on electrophoresis in SDS.
- (6) 5. Draw a Ramachandran map, label the axes properly, and indicate on the map the conformational location of (a) an alpha helix, (b) a beta sheet, (c) collagen.
 2 pts each

(a)

(c)





BCH 4053 -- Hour Test-2

Page 3

(3) 7. What is the structural relationship between L-glycerol-3-phosphate and D-glycerol-1-phosphate?

The same compound

(16) 8. Complete the following table by supplying the missing information on each fatty acid. Be sure to show the double bonds in the correct *cis* or *trans* orientation.

1 pt each Abbreviat ion.	Common Name	Systematic Name	Structure	Omega designatio n
9,12 -C ₁₈₂	linoleic acid	9,12- octadecadieno ic acid	H ₃ C(CH ₂) ₃ (CH ₂) ₆ COOH	ω-6
9,12,15- C _{18:3}	a-linolenic acid	9,12,15- octadeca- trienoic acid	СООН	ω-3
9-C _{18:1}	oleic acid	9 octa- decenoic acid	Соон	ω-9
5,8,11,14- C _{20:4}	arachidonic acid	5,8,11,14- eicosatetraeno ic acid	COOH	ω-6

(6) 9. Phosphatidyl ethanolamine and lysophosphatidyl ethanolamine form different types of aggregate structures. Describe the different structures (words or diagram), and explain what structural difference between the two lipid molecules accounts for this difference.

2 pts each:

PE forms bilayer structures (or lamellar sheet structures) lysoPE forms spherical micelles PE has two hydrocarbon chains per molecule, lysoPE has only one.

(Okay if bilayer and spherical micelle structure are drawn)

(8) 10. **Circle** the following lipids which are negatively charged at pH 6, and **underline** those that contain a nitrogen atom.

0.5 pts each mark on each structure phosphatidyl choline phosphatidyl glycerol cholesterol



(9) 11. Distinguish between integral and peripheral membrane proteins in terms of

 (a) types of solutions used to extract them from membranes.
 3 pts each part, 1.5 each subpart integral—organic solvents and detergents peripheral—high salt, chelating agents, mild detergents

(b) forces by which they are attached to membranes.

integral—hydrophobic attachment to hydrocarbon center of lipid bilayer peripheral—ionic, polar, and hydrogen bonding

(c) membrane location in the fluid mosaic model.

integral—imbedded in or through the lipid bilayer peripheral—attached to surface at lipid head groups or surface of integral proteins

(6) 12. Four types of lipid-linked anchors are known that attach proteins to membranes. Describe two of them, including the lipid involved and the manner in which the lipid is attached to the protein.

Any 2, 3 pts each

N-myristoylation: amide linkage to N-terminal glycine

fatty acyl thioester: several fatty acids (myristate, palmitate, stearate, oleate) linked to cysteine SH

polyprenyl thioether: farnesyl or geranylgeranyl ether to cysteine SH (Cys carboxy is methylated)

phosphatidyl inositol: glycosyl linkage between inositol and oligosaccharides, which is connected through ethanolamine to carboxyl terminal group of protein