BCHM 463 **Your Name:** Key

Biochemistry and Physiology **ID #:**

Exam I, October 9, 2002 **Prof. Jason Kahn**

You have 50 minutes for this exam.

Exams written in pencil or erasable ink will not be re-graded under any circumstances.

You may use a calculator for this exam. No other study aids or materials are permitted.

Generous partial credit will be given, *i.e.*, if you don't know, guess.

Explanations should be concise and clear. I have given you a lot more space than you should need.

N = 97 here
+3 DSS
+1 email
~~1 typo~~

Honor Pledge: Please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

[Should give +3 points for this]

1. "Bioinformatics" (20 pts):

(a; 15 pts) Homology search programs use similarity matrices like BLOSUM62 in assessing the likelihood that two sequences are homologous. List and briefly describe three elements, other than the simple fact of amino acid identity, that enter in to assigning a "score" for a given alignment. In other words, what aspects of our knowledge of protein structure and synthesis are embodied in the matrix and alignment procedures?

5 points each; 2 for identification + 3 for explanation – examples include

– Amino acid similarity - comparison of physical properties, e.g. hydrophobicity. Leu is more likely to be replaced by Ile than by Glu.

– Gaps – penalties are assessed for sequence gaps but they must be considered if there is homology on each side

– Genetic code - a single-nucleotide substitution is more likely than a change that requires ~~two~~ two or three mutations

– Amino acid prevalence – identity between rare amino acids is less likely to arise by chance than between common aa's

– Observed substitution frequencies and helix/sheet preferences - ~~description~~ empirical definition of amino acid similarity.

– Relationships among families of proteins, though this doesn't really relate to ~~not~~

(SP)

(b; 5 pts) A homology search is usually the first thing one does upon isolating a new gene/protein from a genetic screen. What is the most important thing one hopes to find out by doing this? Name a frequently-used homology search program.

+3 for function or structure or both

One is looking for the likely biochemical function of the new gene, as well as its structure if possible. Is it a kinase? An aldolase? Does it hydrolyze ATP?

+2 BLAST, FASTA

2. Hemoglobin (25 pts):

(a; 10 pts) When bicarbonate HCO_3^- is produced in the red blood cell, it equilibrates across the membrane with the blood plasma. Charge neutrality requires that another anion must come in if bicarbonate goes out. This anion is chloride, Cl^-. Based on your appreciation of hemoglobin as a finely tuned oxygen delivery machine, do think that chloride will (a) promote O_2 release or (b) promote O_2 binding, and why? Given your answer, to which state of Hb (R or T) must the chloride bind more tightly?

[should have been 8 points]

(+3) for idea that it's happening in tissues

Bicarbonate is produced from CO_2 in tissues with active metabolism. Therefore O_2 is needed in that area, and Cl^- should promote oxygen release. (+2) (+2)

In order to promote release, Cl^- must bind the T state preferentially. (+3)

+6 altogether for consistent but wrong biochem answer

+7 for biochemically sensible answer w/out physiology component

Also - Cl^- influx should ↓ pH by driving $H_2O + CO_2 \rightarrow HCO_3^- + H^+$

Cl^- essentially replaces CO_2 in structure to maintain T state

CO_2 does not bind at heme! Nor does Cl^-!

JDK

actually 8 (oops) - doesn't affect the problem

10 [should have left it 12]

(b; 12 pts) The Bohr effect refers to the release of protons upon O_2 binding to Hb. Much of the Bohr effect is due to a change in the pK_a of the C-terminal histidine of the ß subunit (His 146ß). The pK_a of this histidine is about 7.1 in the T state. Given this fact, calculate what percentage of the total His146ß is protonated at pH 7.4. In order for it to contribute to the Bohr effect, must the pK_a of this residue go up or down upon conversion to the R state, and why? What sort of interaction in the protein could cause this effect?

+1 [$pH = pK_a + \log \frac{[A^-]}{[HA]}$ In this case $HA = HisH^+$

$7.4 = 7.1 + \log \frac{[His]}{[HisH^+]}$, so $\frac{[His]}{[HisH^+]} = 10^{0.3} = 1.995$] +2

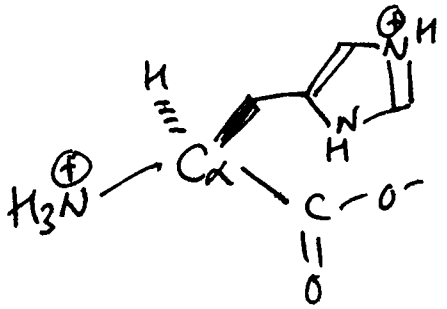
protonated fraction $= \frac{[HisH^+]}{[His]+[HisH^+]} = \frac{[HisH^+]}{1.995[HisH^+]+[HisH^+]} = \frac{1}{3}$ +2

+1 [To release protons, $HisH^+$ must become a stronger acid,
+1 [and therefore pKa must decrease. (Actually goes to ~7.1)

+3 for either explanation (or reverse, to be consistent with prev. answer) [The drop in pKa could be caused by burial of His in a more hydrophobic region (preferring neutral species), or withdrawal of a stabilizing salt bridge or anion (actually Asp 94/ß)

(c; 5 pts) Draw the structure of the dominant prototropic form of the plain amino acid histidine at pH 4, including stereochemistry at C_α.

At pH 4 Histidine will be protonated on the side chain



+1 for amino acid
+1 for protonated
+1 for stereochemistry
+2 for correct side chain
[+1 for ~~minor error~~ if 3° amino error]

SP

3. Ramachandran Angles and Secondary Structure (25 pts):

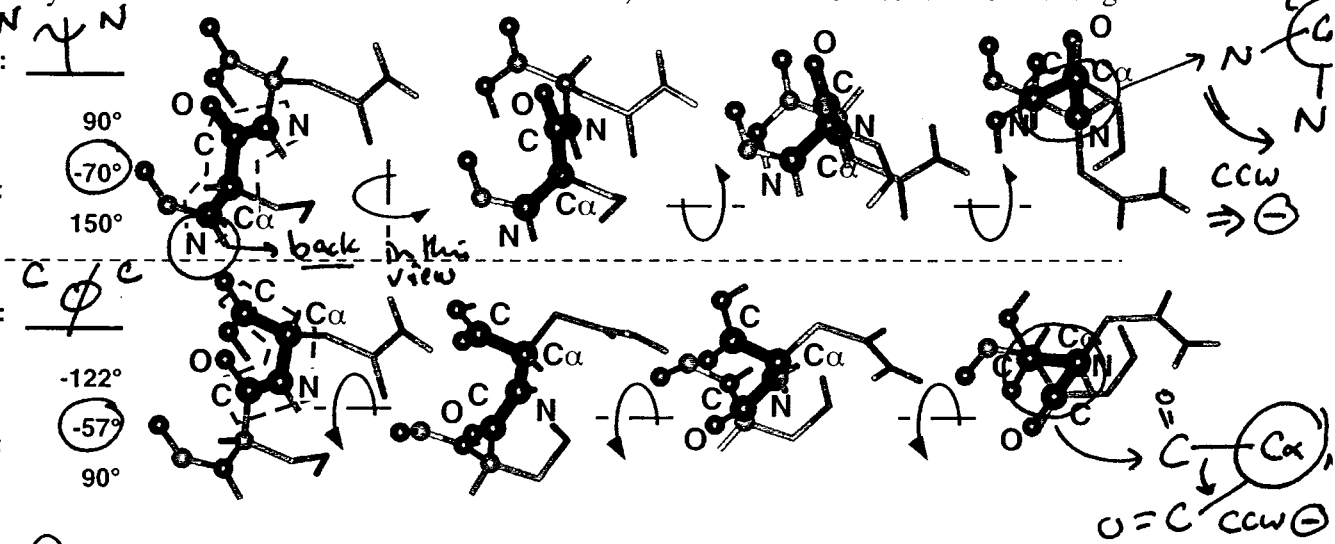
(a; 16 pts) The diagram below shows a peptide fragment in which all of the amino acids have the same Phi (Φ)and Psi (Ψ) angles. The overhead shows the same thing in color. All eight images are the same peptide in different orientations, with gradual rotations from left to right in each row. In the blank on the left, write which angle is defined by the four atoms represented by the thick bonds and boxed in the dashed line, and circle the correct value of that angle.

+4 Angle: N–Cα–N

+4 Circle Value: 90° (-70°) 150°

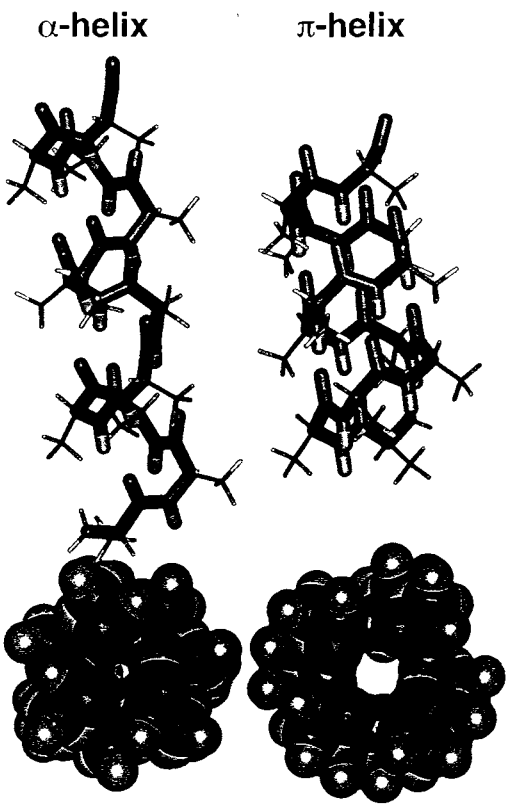
+4 Angle: C–Φ–C

+4 Circle Value: -122° (-57°) 90°



indicates rotation of the picture about the indicated axis, in the direction of the arrow

The fragment above is part of a conceivable secondary structure called the "π-helix," which is shown on the right in comparison to the familiar α-helix.



(b; 4 pts) The Φ and Ψ angles for the π-helix are in an allowed part of the Ramachandran diagram. What does it mean to be in an allowed region?

This means that there are no local steric clashes which would prevent the peptide from assuming the specified Φ/Ψ angles.

+2 for idea of allowed conformation

+2 for idea it's because of steric

SP

(c; 5 pts) The π-helix is not, however, observed in real proteins. Based on your understanding of the principles of protein structure, why not?

The π-helix has a hole down the middle. (+2)

That means it would lose enthalpic van der Waals stabilization, or else the channel might be occupied by highly ordered H_2O molecules. (+3)

4. ß Sheets: The Good, the Bad, and the Ugly (30 pts):

(a; 14 pts) Name a prion disease. Briefly describe what is known about the sporadic appearance and about the transmission of prion diseases. What is (some of) the evidence that the infectious particle has no nucleic acid component? Why are prions somewhat surprising in terms of how we normally think about protein folding?

(+2) Mad cow disease, BSE, vCJD, Creutzfeldt-Jacob disease, Kuru, etc

Prion diseases are due to variant, pathological protein conformations. They can appear sporadically if one or several or many proteins spontaneously convert to the variant structure, after which the converted protein nucleates conversion of other, normal, proteins to the variant conformation. Alternatively, the variant form can be introduced from outside, by direct injection (in experimental animals), or by eating parts of infected animals that have the protein (as in BSE).

(+2 for role of variant formation; +2 for conversion of native by variant; +2 for modes of transmission; ↑+8)

The infectious agent is unaffected by nucleases or UV irradiation, which should destroy nucleic acid. No virus has been found. (+3)

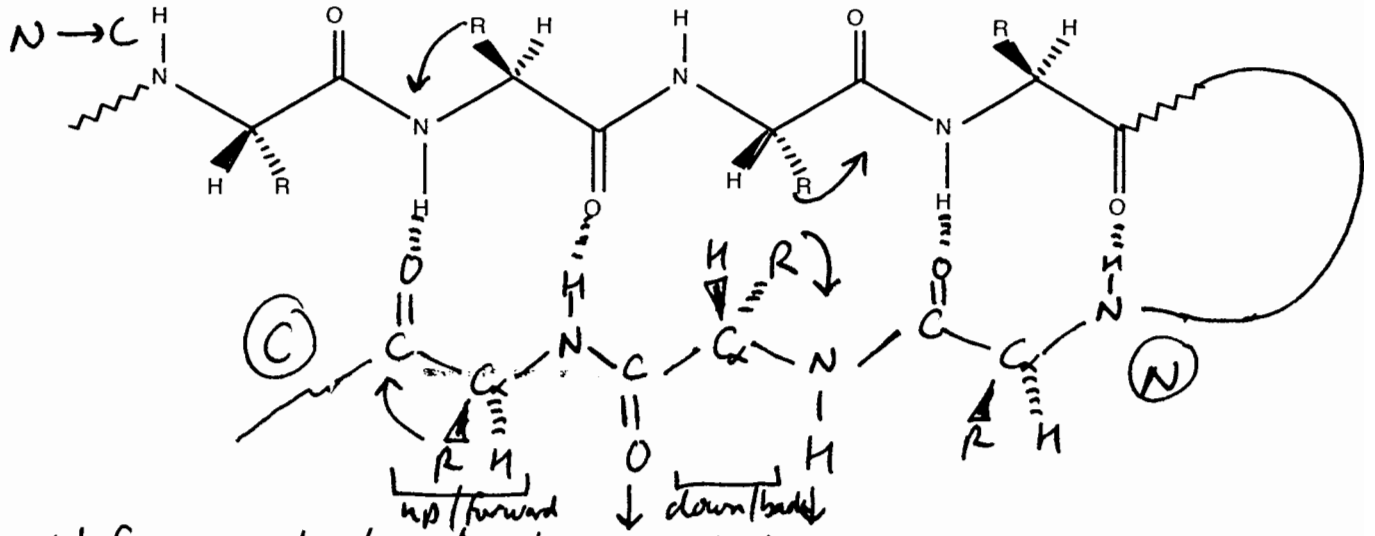
-Usually we think of proteins as having one specific native state, not multiple stable conformations. In addition, the species barrier suggests there must sometimes be multiple variant conformations (+3)

It's not a mutation! It's conformation (although probably a genetic component to susceptibility)

[+1 for fibrils if other pts. missing]

JDK

(b; 10 pts) Draw in three residues of the antiparallel β-sheet partner below the extended polypeptide chain drawn here. Include Cα stereochemistry and backbone hydrogen bonds, and indicate where the next strand (i.e. if you were to draw a third one) would H-bond. Indicate side chains with R. Indicate the N terminus and the C terminus of the strand you draw.



+1 for correct stereochemistry
+2 for drawing a peptide
+2 for hydrogen bonding
+1 for next strand
+1 for N and C termini
+1 for neatness

(c; 6 pts) What are the steric interactions that cause the βsheet to be pleated? On your diagram above, indicate which Cα's will be pushed forward out of the plane, and which ones will be back.

(+3) Side chain repulsions — the sterics force the side chains to be essentially perpendicular to the plane of the sheet. See arrows above.

(+3) for correct directions

Score:	1.	"Bioinformatics" (20 pts):	_____
	2.	Hemoglobin (25 pts):	_____
	3.	Ramachandran Angles and Secondary Structure (25 pts):	_____
	4.	ß Sheets: The Good, the Bad, and the Ugly (30 pts):	_____
		Total: out of 100	_____

(SP)