**BCHM 463** **Your Name:** Key

**Biochemistry and Physiology** **ID #:**

**Exam II, March 19, 2003** **Prof. Jason Kahn**

You have 55 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.

N = 9
N = 53 Σ = 62
+1 email

Hill equation: $Y_{O_2} = \frac{(pO_2)^n}{(p50)^n + (pO_2)^n}$

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

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."

## 1. Hemoglobin allostery and oxygen transport (35 pts):

(a; 10 pts) Briefly describe three steps, in terms of changes in protein and heme structure, in the allosteric induction of the R state of hemoglobin upon binding a molecule of $O_2$. What one word describes the shape of the resulting binding curve?

- $O_2$ binds to $Fe^{+2}$ in heme

(+1) → sigmoidal or cooperative

- ~~Heme Rotation~~ Leads to movement of $Fe^{+2}$ that flattens out domed T-state heme to flat R state heme

- This has the effect of pulling on the distal histidine ligand of the $Fe^{+2}$.

- Leads to a change in the position of helix F

- And that leads to a change in the inter-subunit interfaces which promotes conversion of neighboring subunits into the R state.

(+3 each up to three = +9 total)

(b; 25 pts: 5 pts each) For each of the following ligands, indicate whether it binds better to the R state or the T state of hemoglobin, and briefly describe the significance of the preference to physiological oxygen transport.

$O_2$ (Circle one: (R) T ) Significance: (+1)

Leads to cooperative binding (+2) of $O_2$, as subsequent $O_2$ molecules bind to Hb that's already ~~paid~~ in the R state. Therefore more efficient (+2) delivery of $O_2$ to tissues.

$H^+$ (Circle one: R (T) ) Significance: (+2)

Bohr effect - binding of protons ~~Ed~~ induces T state, so low pH in tissues (from $CO_2$) leads to ↑ release of $O_2$. (+3)

$CO_2$ (Circle one: R (T) ) Significance: (+1)

Carbamate formation allows Hb to carry (+2) $CO_2$ to the lungs, and also stabilizes T state → ↑ release of $O_2$, and also contributes via Bohr effect. (+2)

NO (Circle one: (R) T ) Significance: (+1)

- Hb carries •NO and ∴ protects it from decaying. Conversion to T state leads to release of •NO → ↑ vasodilation in active tissues. (+1) (+3)

BPG (Circle one: R (T) ) Significance: (+2) (+1)

(+2) - Decreases $O_2$ affinity relative to stripped Hb into useful range

(+2) - Allows for changing $O_2$ affinity in response to altitude

## 2. Protein folding and prions (25 pts):

(a; 9 pts) What is the molten globule in protein folding? How does the activity of chaperones in unfolding molten globules enhance the overall rate of folding to the native state?

(+2)

- The molten globule is a folding intermediate with a hydrophobic core, substantial (+2) secondary structure, but (+2) incorrect/nonnative tertiary structure. Unfolding of the molten globule can be rate-limiting for folding of some proteins to the native state.
- Chaperones accelerate the slow process of unfolding/disrupting the molten globule and allow the protein to "try again" to fold into the native state. (+3)

(b; 8 pts) Name a protein folding disease. Give two possible general causes for disease arising from protein folding defects.

(+2) for any one: BSE, vCJD, Alzheimer's, Type II diabetes, kuru, Huntington's disease, cystic fibrosis

- General causes for disease can be

(+3) each for any two:

1. Loss of function of protein product (e.g. diabetes, CF).
2. Toxic effects of aggregated protein, for example leading to immunological attack or apoptosis (Alzheimer's plaques?)
3. Toxic effect of possible intermediates along the aggregation pathways (e.g. possible membrane pores).
4. ?

(c; 8 pts) Give two rationales for the prevalence of independently folding 100-200 residue domains in proteins. How might you identify domain boundaries, either experimentally or by sequence analysis (pick one)?

(+3) - Efficient use of "coding capacity" – it's the surface of the protein that does the work, no need to waste most of the amino acids by burying them.

(+3) - Ability to evolve new functions by mixing + matching functions encoded by domains, e.g. SH3 domains, ATP binding folds, etc.

(+2 for either) - Identify domains by increased sequence conservation (BLAST does this automatically) within a protein. –OR– Light trypsin digestion to preferentially cut inter-domain linkers

## 3. Protein sequence analysis and evolution (25 pts):

(a; 6 pts) The sequence below shows three segments of a protein region that binds retinol, a large hydrophobic ligand. Do you think the binding region is an α-helix or a β-sheet? Explain your reasoning. (Exaggerated from the actual sequence given by Branden and Tooze.)

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| … Ile | Ala | Val | Glu | Phe | Ser | Val | Asp | … |
| … Met | Ser | Ala | Thr | Val | Lys | Gly | Arg | … |
| … Ala | Asp | Met | Val | Trp | Cys | Phe | Thr | … |

(+3) Alternating bulky hydrophobics and polar/charged residues

(+3) suggests a β-sheet, because the alternating sides would allow the h-phobic side to face in toward the ligand and the polar/charged side to be on the surface.

(b; 5 pts) Which is more conserved in evolution, primary sequence or tertiary structure? Why?

(+2) 3° structure is more conserved because it is the 3° structure that encodes (+3) function.

(c; 9 pts) How are protein sequences used in constructing phylogenetic trees? Why does the phylogeny of a protein sometimes not match up with the phylogeny of the organisms it inhabits?

(+3) | - Sequences are aligned to identify sequence identity and similarity, and then the phylogenetic tree that minimizes the number of nucleotide changes needed to give the observed pattern of sequences.

(+3 for anything reasonable)

(+3) | If the protein appears to have dropped in from outer space it may be due to horizontal transfer of genetic information, eg. DNA being carried in by viruses or transposons.

(d; 5 pts) How can the analysis of protein families improve homology searches? Name a program that extends BLAST in this way.

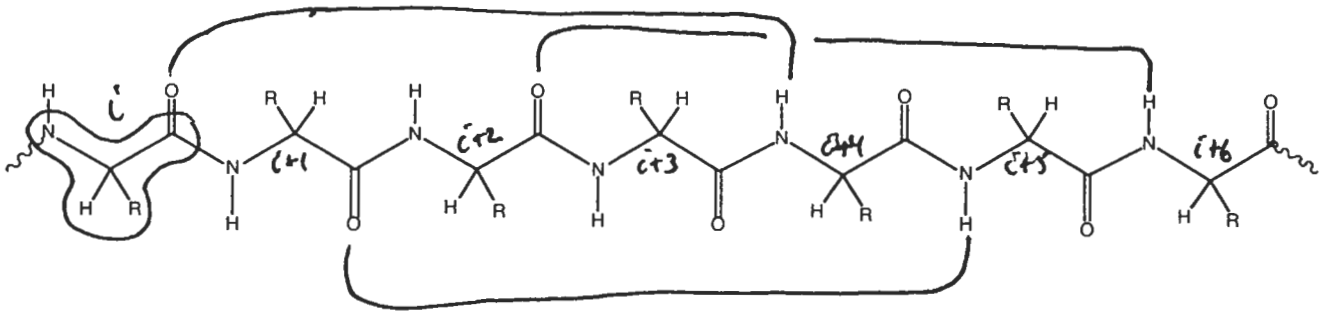
(+3) - Looking at family members allows us to detect weak homology by finding proteins that are related to the results of a first homology search. i.e. it may be clear that A is related to B and C, but only by looking at B and C is it clear that A and D are part of the same family.

(+2) - PSI-BLAST

not needed | - Iterated Position Specific

## 4. Secondary structure (15 pts):

(a; 6 pts) On the extended peptide representation below, diagram the hydrogen bonding pattern of the α-helix. In other words, draw in which three carbonyls are H-bonded to which three amides.



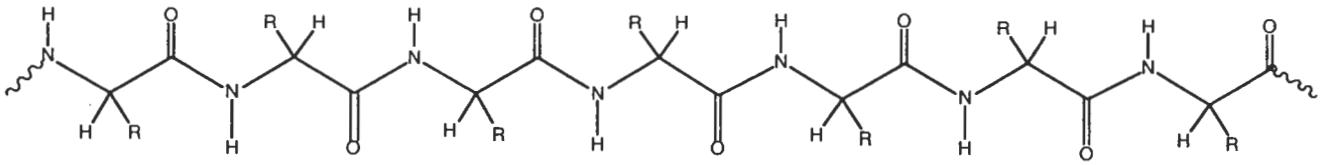

i → i+4 H-bonding

+2 for each correct H-bond = +6

+3 total for consistent i → i+3 or i → i+5

+2 total for any N-H ··· O=C H-bond

If you mess up, try again here:

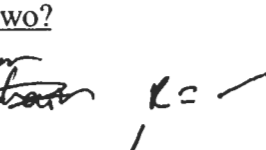
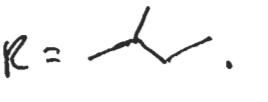


(b; 5 pts) What is the number of residues per turn for the α-helix? The $i \rightarrow i \pm$ (some number) H-bonding pattern you diagrammed in (a) connects amino acids that are one above another along the direction of the helix axis. How can this be consistent with a non-integral number of residues per turn?

(+2) 3.6 residues / turn

(+3) The "leading edge" carbonyl of residue $i$ H-bonds to the "trailing edge" of residue $i+4$, therefore the points directly above and below each other represent ~~[illegible]~~ less than a full four residues.

(c; 4 pts) Why does leucine have a greater propensity to be found in the α-helix than isoleucine, whereas Ile has the greater β-sheet propensity of the two?

(+2) Leu has an unbranched ~~side chain~~ β carbon R =  , whereas Ile is branched at C-β- R =  .

(+2) The β-sheet has more room near C-α, so bulky side chains, especially if β-branched, encounter less steric hindrance. Leu packs better on the more crowded surface of the α-helix.

| | | |
|---|---|---|
| Score: | 1. Hemoglobin allostery and oxygen transport (35 pts): | _____ |
| | 2. Protein folding and prions (25 pts): | _____ |
| | 3. Protein sequence analysis and evolution (25 pts): | _____ |
| | 4. Secondary structure (15 pts): | _____ |
| | **Total: out of 100** | _____ |