

CHEM-342 Introduction to Biochemistry
Final Examination - Individual (Part I)
Tuesday, 27 May 2008
7:00 – 8:45 PM
H. B. White - Instructor

Your Name _____

*We are not afraid to follow truth where ever it
may lead, nor tolerate error so long as reason
is left free to combat it.*

Thomas Jefferson

Class average 61.9, Class range 32-84/90, N=26

Important - Please read this before you turn the page.

- For the first hour, this is a closed book examination. From 8 – 8:45 PM you may refer to your course notes and materials.
- This examination will assess your learning, problem-solving skills, and ability to communicate clearly. It is intended to be challenging even to the best students in the class. Writing reflects how you think. Among the “right answers” I will read, some will be better than others because they show greater depth of understanding, avoid extraneous or inaccurate information, provide a more logical structure, use appropriate examples, and choose words with precision. Better quality answers will receive higher marks. Therefore organize your thoughts before you write or draw. Strive to write not that you may be understood, but rather that you cannot possibly be misunderstood. Stream of consciousness answers are rarely well organized or clearly presented.
- This examination emphasizes work done in this course since Spring Break; however, knowledge is not so conveniently compartmentalized. Therefore, you should feel free to use any relevant example from your experience, if it is appropriate.
- There are 8 pages to this part of the examination (counting this cover page and the information sheet at the back). Please write your name on each page. Feel free to use the backs of pages, if you need more space.
- Part I (90 points) This individual part of the examination, includes 10 problems and short essay questions.
- Part II (30 points) The group part of the examination will require you to deal with new information collaboratively.
- If you complete Part I early, you may leave the room and move to 205 Brown Lab where the Group Part of the examination will begin about 9 PM.
- You may refer to your notes, course reader, handouts, or graded homework assignments after the first hour of the examination.
- Attempt to draw a picture or diagram as part of your answer to every question.
- Graded examinations may be picked up Thursday morning, 29 May.
- Have a productive and safe summer.

Short Answer: (3 points each, 27 points total): The following require brief but well-worded answers that concisely and accurately answer the question in one or two sentences.

1. Linus Pauling hypothesized that sickle cell anemia was a disease of the hemoglobin molecule and not of the red blood cell several years before he and his coworkers produced molecular evidence to test and support his hypothesis.
 - A. Specifically, what led Pauling to deduce that hemoglobin in sickled cells was different?

 - B. What experimental evidence did Pauling et al. (1949) provide to support the hypothesis?

2. Building on Pauling's work, Vernon Ingram set about to identify the exact chemical difference between normal human hemoglobin and sickle cell hemoglobin. His strategy was to make a complex problem simpler.
 - A. Conceptually, what did Ingram do to simplify the problem?

 - B. What difference did Ingram find?

3. Howard Dintzis, having worked with Ingram in the same laboratory in Cambridge, England, later used Ingram's methods to solve a fundamental problem in biochemistry.
 - A. What problem did Dintzis solve?

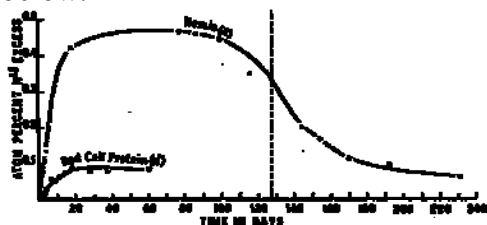
 - B. What did Dintzis conclude?

4. Intrigued by the high frequency of the sickle cell gene in parts of Africa, Anthony Allison hypothesized that natural selection must be involved.
 - A. By what logic did Allison deduce that natural selection affected the frequency of the sickle cell gene?

 - B. What experimental evidence did Allison provide in support of his hypothesis?

5. Howard Dintzis was not the first biochemist to study hemoglobin synthesis with isotopically labeled amino acids. Fifteen years earlier, David Shemin and David Rittenberg used ^{15}N glycine as a precursor and discovered something quite unexpected.
- A. What did Shemin and Rittenberg discover that was unexpected?

6. (12 Points) If David Shemin had decided to feed rabbits uniformly-labeled ^{15}N -glycine, his result would have been qualitatively similar to the results he got on himself shown below.



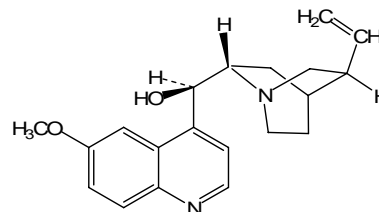
- A. (6 points) Imagine an experiment like Shemin's in which rabbits (instead of humans) were fed ^{14}C -glycine (instead of ^{15}N -glycine). Draw a figure above depicting the amount of ^{14}C in hemin and in hemoglobin as a function of time.
- B. (6 points) Imagine an experiment like Shemin's in which rabbits (instead of humans) were fed ^{14}C -leucine (instead of ^{14}C -glycine). Draw a figure below depicting the amount of ^{14}C in hemin and in hemoglobin as a function of time. (You may make comments to explain your reasoning.)

7. (21 Points) Food scientists have an interest in food flavor. Frequently, the breakdown of proteins generates bitter-tasting peptides. Because hemoglobin is an abundant protein in blood, significant amounts of it remain in meat products and could affect taste. Pepsin hydrolysis of beef hemoglobin yields several bitter peptides. Among them is a fragment of the β -globin chain including amino acid residues 32 through 40 shown below. Its bitterness at 250 μ M is equivalent to 73 μ M quinine, a very bitter compound. [Auloes-Dufau et al. (1995) "Bitter peptide from hemoglobin hydrolysate: Isolation and characterization." *FEBS Lett.* **364**, 115-119.]

V-V-Y-P-W-T-Q-R-F

- A. (5 points) If one were separating pepsin peptides of bovine hemoglobin by ion exchange column chromatography and monitoring the peptides by their UV absorption, would you expect this peptide to absorb strongly or weakly at 280 nm? Explain your answer.
- B. (5 Points) After studying many peptides scientists established a basis for predicting bitterness known as the "Q-Rule" based on hydrophobicity and size. Generally bitter peptides are hydrophobic and have a molecular mass less than 6000. [Ney (1979) *ACS Symposium Series* **115**, 149-173] Based on the Q-Rule, would you suspect that the above peptide would be bitter? Justify your answer.
- C. (6 Points) Draw the chemical structure of the C-terminal dipeptide R-F (argininylphenylalanine) [Amino acid structures are available on the last page.]

- D. (5 Points) What do you know about quinine related to material in CHEM-342?



8. (10 Points) Please defend or refute one of the following propositions related to Dr. Gingrich's visit
- A. Because informed consent is not the same in the military as it is in civilian life, the experiment done by Allison could be probably still be done with enlisted Army volunteers.
 - B. Because informed consent did not apply to prisoners in the 1940's, much of what we know about malaria was learned by deliberately infecting prisoners and studying what happened to them.
 - C. We can expect a major epidemic of malaria in Myanmar (formerly Burma) in the next month or two.

9. (10 Points) The following is the first paragraph of an article by Kihm et al. (2002) entitled, "An abundant erythroid protein that stabilizes free α -haemoglobin," and published in the British journal *Nature* **417**, 758-763.

The development of red blood cells (erythrocytes) is distinguished by high-level production of the oxygen carrier, haemoglobin A (HbA), a heterotetramer of α - and β -haemoglobin subunits. HbA synthesis is coordinated to minimize the accumulation of free subunits that form cytotoxic precipitates. Molecular chaperones that regulate globin subunit stability, folding or assembly have been proposed to exist but have never been identified. Here we identify a protein stabilizing free α -haemoglobin by using a screen for genes induced by the essential erythroid transcription factor GATA-1. Alpha Haemoglobin Stabilizing Protein (AHSP) is an abundant, erythroid-specific protein that forms a stable complex with free α -haemoglobin but not with β -haemoglobin or haemoglobin A ($\alpha_2\beta_2$). Moreover, AHSP specifically protects free α -haemoglobin from precipitation in solution and in live cells. AHSP-gene-ablated mice exhibit reticulocytosis and abnormal morphology and intracellular inclusion bodies that stain positively for denatured haemoglobins. Hence, AHSP is required for normal erythropoiesis, probably acting as a block to deleterious effects of free α -haemoglobin precipitation. Accordingly, AHSP gene dosage is predicted to modulate pathological states of α -haemoglobin excess, such as β -thalassaemia.

The existence and role of AHSP has not yet appeared in textbooks. A textbook author thinks it would be worth including this information in the next edition of her textbook as a figure. In the space below, make a sketch of where AHSP fits into the production and assembly of hemoglobin A.

10. (10 Points) Among the rare inherited variants of hemoglobin there are examples of ones that increase or decrease the affinity of the hemoglobin for oxygen. People whose Hb has an abnormally high affinity for oxygen tend to have polycythemia – an abnormally high number of RBC/ml blood. People whose hemoglobin has an unusually low affinity for oxygen tend to have anemia – an unusually low number of RBC/ml blood. Doctors used to try and correct these unusual RBC levels, but now realize they are the result of a physiological compensation mechanism. The alterations in RBC allow normal levels of oxygen to reach the tissues in spite of the unusual hemoglobin properties. (Problem written by Dr. Deborah Mowshowitz, Columbia Univ.)

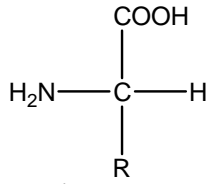
A. (2 Points) People with high affinity hemoglobin (like Hb Ranier) probably have polycythemia because they need to adjust the ability of the blood to:

- i. pick up oxygen in the lung
- ii. release oxygen in the tissues
- iii. release oxygen in the lungs
- iv. pick up CO₂ in the tissues
- v. release CO₂ in the lungs
- vi. release CO₂ in the tissues.

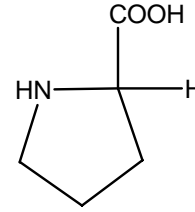
B. (2 Points) People with low affinity hemoglobin (like Hb Seattle) probably have anemia because they need to adjust the ability of the blood to

- i. pick up oxygen in the lung
- ii. release oxygen in the tissues
- iii. release oxygen in the lungs
- iv. pick up CO₂ in the tissues
- v. release CO₂ in the lungs
- vi. release CO₂ in the tissues.

C. (6 Points) Explain briefly how the polycythemia or anemia helps maintain normal function in both cases.



Basic Structure of the amino acids found in proteins



Proline (P)
 an Imino Acid

Various R-Groups associated with particular protein amino acids

