

Introduction to Biochemistry
Final Examination - Individual (Part I)
Thursday, 24 May 2012
3:30 – 5:30 PM, 206 Brown Lab
H. B. White – Instructor

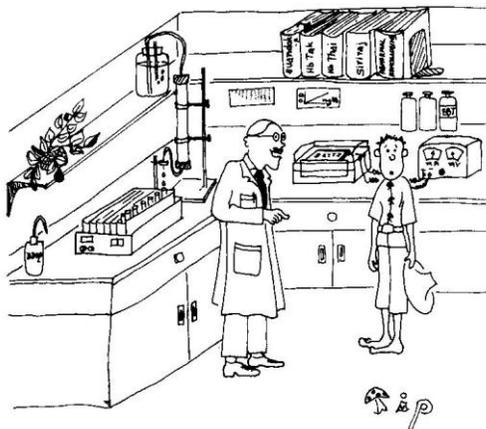
Your Name _____

Mean \pm SD = 72.3 \pm 17.6
Range = 23 – 99 out of 112 points
N = 27

112 Points

Important - Please read this before you turn the page.

- For the first hour (3:30 – 4:30PM), this is a closed-book examination. From 4:30 – 5:30PM you may refer to your course notes and materials. Textbooks and electronic devices are not permitted. Turn off cell phones.
- 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). There is also a worksheet distributed separately. Please write your name on each page. Feel free to use the backs of pages, if you need more space.
- Part I (130 points) This individual part of the examination, includes 15 short answer, problems, and 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 5:30 PM.
- You may refer to your notes, course reader, handouts, or graded homework assignments after the first hour of the examination and for the group part of the examination.
- Attempt to draw a picture or diagram as part of your answer to every question.
- Have a productive and safe summer.



Congratulations!! Your hemoglobin is special, we'll name it after you.

Questions 1-5, Short Answer: (4 points each, 18 points total): The following questions 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 Max Perutz's 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 (who subsequently worked in both Perutz's and Pauling's labs) 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?

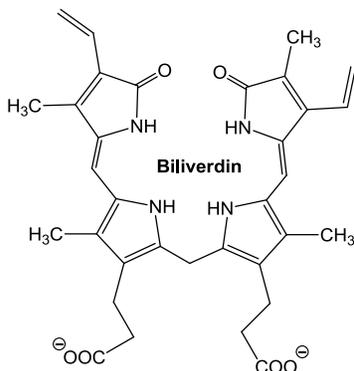
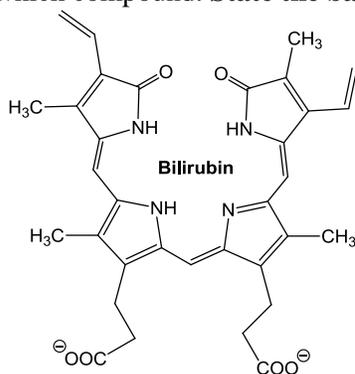
 - B. What experimental evidence did Allison provide in support of his hypothesis

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.

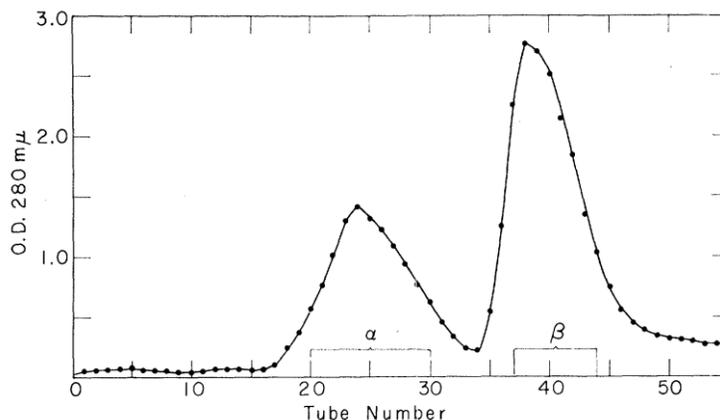
A. What did Shemin and Rittenberg discover that was unexpected?

6. **Seminar** (5 points) You were asked to attend two seminars during the semester. Pick one and describe something about it even though it may have been pretty advanced.

7. (6 points) Bilirubin and biliverdin, drawn below, are breakdown products of heme. They provide the array of colors seen in a bruise. Structurally, they differ only by the reduction of a double bond between the middle two pyrrole rings. Their two carboxyl groups appear virtually identical. However, the pKa values for each molecule are different. In one case the values are 4.2 and 4.9 while in the other they are 3.9 and 5.3 [*J. Biol. Chem.* **271**, 2397 (1996)]. **Predict** which set of values goes with which compound. **State the basis** for your prediction.



8. (11 points total) In Dintzis' experiment, he separated the α and β globin chains of rabbit hemoglobin using carboxymethyl-cellulose column chromatography. The elution profile Dintzis observed is shown below.



- A. (6 points) What is a carboxymethyl-group? What is cellulose? What is carboxymethyl-cellulose?
- B. (5 points) Pauling et al. (1949) determined the isoelectric point for HbA which is composed of two α and two β subunits. What would you predict about the isoelectric points of the separated α and β subunits based on the elution profile from carboxymethyl cellulose column above? Explain your reasoning.

9. (10 points) The amino acid sequences of rabbit alpha and beta hemoglobin chains are given on a separate worksheet the one-letter abbreviations for amino acids. The alpha and beta chains are paralogous, which means they resulted from an ancient gene duplication. This explains their similar size and folding and also the remnants of their ancestral amino acid sequence. It turns out that one of the tryptic peptides for rabbit alpha globin is identical to one from rabbit beta globin. Identify that tetra peptide and draw its complete covalent structure below.
10. (10 points) The secondary structure of hemoglobin is about 70% alpha helix, a hydrogen-bonded structure deduced by Linus Pauling from first principles. Draw a peptide bond and show how that peptide bond hydrogen bonds to other peptide bonds in an alpha helix.

11. (10 points) The question that proved to be the most difficult on the Pauling-Ingram quiz was #18 that follows. Draw a graph of a titration of acetic acid (pH vs equivalents of base) using the information in the question. Based your graph show which of the four choices is correct. No credit without a figure and explanation.

Pauling et al. (1949) determined that, near its pI, hemoglobin dissociates ~13 protons per molecule with a 1.0 increase in pH. Consider a monoprotic acid like acetic acid. On average, how many protons per molecule would dissociate in going from a pH = pKa = 4.76 to a pH of 5.76?

- A. 1.0 B. 0.9 C. 0.5 D. 0.4

12. (10 points) Shemin and coworkers repeated their study of the incorporation of ¹⁵N-labeled glycine into hemin on a person who had sickle cell anemia [*J. Biol. Chem.* **179**, 468 (1949)]. Draw in the results that Shemin and Rittenberg got. Qualitatively and quantitatively, how does this figure differ from normal? How can these differences be explained?

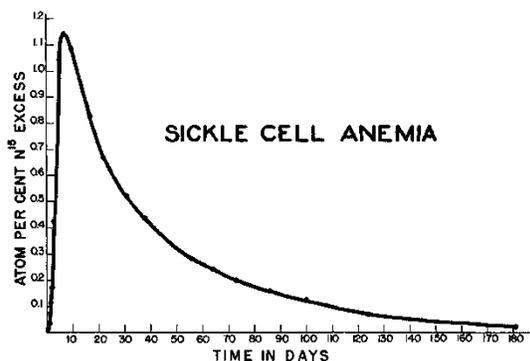


FIG. 4. ¹⁵N concentration in hemin after feeding ¹⁵N-labeled glycine for 2 days

13. (12 Points) Pauling and coworkers observed that heterozygous individuals for the sickle cell gene produce a 40:60 ratio of HbS to HbA, rather than a 50:50 ratio. The following describes an in vitro (cell-free in a test tube) experiment which gave a similar but much more striking result [Schreier et al., *European Journal of Biochemistry*, **34**, 213 (1973)].

A cell-free protein-synthesizing system, reconstituted with various components (ribosomes, aminoacylated tRNAs, initiation factors, etc.) from mammalian sources, is strongly dependent on added messenger RNA. mRNAs isolated from duck reticulocytes (A) are translated into duck hemoglobin chains by this system. Likewise, mRNAs from rabbit reticulocytes (B) are translated into rabbit hemoglobin chains. When the system is presented simultaneously with duck and rabbit mRNA (A+B) at equal and saturating concentrations, rabbit hemoglobin synthesis occurs almost exclusively even though the maximal rate of hemoglobin synthesis is relatively unaffected whether the mRNAs are employed separately or in combination.

A. (6 points) Construct a table that displays the results described.

B. (6 points) Provide a simple pictorial model to explain what appears to be happening on the molecular level.

14. (10 points) A group of CHEM-342 students were arguing in the absence of their omniscient tutor:

Alphie "I don't understand. How does hemoglobin 'know' when to release oxygen in the body?"

Betty "Oh that's easy. Whenever it gets to a capillary and encounters high carbon dioxide levels, it lets go."

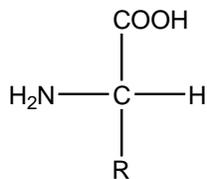
Gammy "It's not the carbon dioxide that does it. It's the lower pH that results from the carbon dioxide dissolved in the blood plasma."

Della "I don't see it that way at all. Oxygen and carbon dioxide are competing for binding. When carbon dioxide concentrations are high, it displaces oxygen where it is needed."

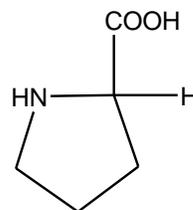
How would you answer Alphie's question? Diagrams welcome.

15. (10 points) Some researchers are thinking about redoing Dintzis' experiment with the benefit of hindsight and they are deciding whether a different labeled amino acid than leucine might be better to use. They have the amino acid sequence and composition of rabbit hemoglobin (on the worksheet provided) and ask you to discuss the relative merits of using **leucine** again versus **lysine or alanine**.

A. Based on the things you know and information provided here, consider the relative merits of the three amino acids and then make a reasoned recommendation. Identify clearly the criteria that you consider to be relevant to your decision.



Basic Structure of the amino acids found in proteins



Proline (P)
an Imino Acid)

Various R-Groups associated with particular protein amino acids

