

Introduction to Biochemistry
Final Examination - Individual (Part I)
Monday, 24 May 2010
7:00 – 8:45 PM
H. B. White – Instructor

Your Name _____

"Ability is what you're capable of doing.
Motivation determines what you do.
Attitude determines how well you do it."

120 Points

Lou Holtz

Ave \pm SD = 73.1 \pm 20.3, Range: 28 – 104 out of 120, N = 28

READ THIS FIRST

- 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 11 pages to this part of the examination (counting this cover page.). Please write your name on each page. Feel free to use the backs of pages, if you need more space.
- Part I (120 points) This individual part of the examination, includes 10 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 8:50 PM.
- You may refer to your notes, course reader, handouts, or graded homework assignments after the first hour of the examination. The group part of the examination is closed book and closed notes.
- Attempt to draw a picture or diagram as part of your answer to every question.
- Have a productive and safe summer.

- 3.** (10 Points) Conceptualize with words and a picture or chemical model how, by changing the pH from 6.5 to 7.5, the net charge on a hemoglobin molecule can go from $\sim +5$ to ~ -8 as described by Pauling et al (1949).

4. (15 points) Fig 1 is from Ingram's first article. It monitors the time course for the digestion of HbA and HbS by trypsin at pH 8. NaOH is added to maintain the pH.

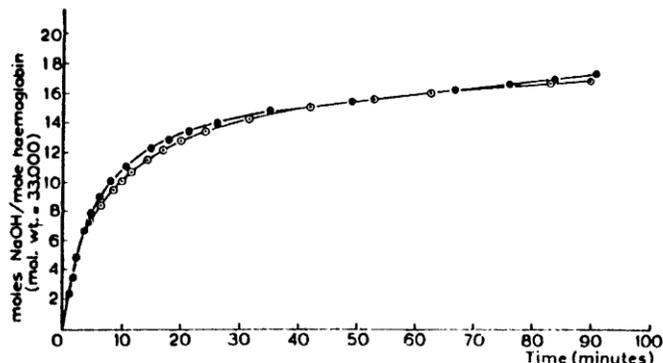


Fig. 1. Course of the trypsin digestion of haemoglobin A and S. \circ --- \circ Haemoglobin A; \bullet --- \bullet Haemoglobin S.

- A. (5 points) Chemically describe in words and structures the reaction catalyzed by trypsin.

- B. (5 points) Explain how there is a net production of protons (H^+) in this reaction.

- C. (5 points) How does the number of equivalents of hydroxide compare to the number of peptide bonds hydrolysed by trypsin?

5. (14 points) When Howard Dintzis wrote his 1961 paper, the amino acid sequences of neither rabbit nor human globins were known. In his second paper, he used the sequence of the human globins to deduce the order of his rabbit tryptic peptides. The complete sequences of human and rabbit globins are now known. They are aligned and displayed below so direct comparisons can be made. (**Note:** The sequences do not fit on a single line and thus wrap to subsequent lines. The single-letter abbreviations for the 20 amino acids are:

A = Alanine	I = Isoleucine	R = Arginine
C = Cysteine	K = Lysine	S = Serine
D = Aspartic Acid	L = Leucine	T = Threonine
E = Glutamic Acid	M = Methionine	V = Valine
F = Phenylalanine	N = Asparagine	W = Tryptophan
G = Glycine	P = Proline	Y = Tyrosine
H = Histidine	Q = Glutamine	

α Globin Sequence

HUMAN VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYFPH
RABBIT VLSPADKTNIKTAWKIGSHGGEYGAEAVERMFLGFPTTKTYFPH

FDSLHGSAQVKGHGKKVADALTNAVAHVDDMPNALSALSDLHAHKLRVDPVNFK
FDFTHGSEQIKAHGKKVSEALTKAVGHLDDLPGALSTLSDLHAHKLRVDPVNFK

LLSHCLLVTLAAHLPAEFTPAVHASLDKFLASVSTVLTISKYR
LLSHCLLVTLANHHHPSEFTPAVHASLDKFLANVSTVLTISKYR

β Globin Sequence

HUMAN VHLTPEEKSAVTALWGKVVNDEVGGEALGRLLEVYPWTQRFFESF
RABBIT VHLSSEEKSAVTALWGKVVNVEEVGGEALGRLLEVYPWTQRFFESF

GDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHLNPKGTFAATLSELHCDKLHVD
GDLSSANAVMNNPKVKAHGKKVLAAAFSEGLSHLDNPKGTFAKLSELHCDKLHVD

PENFRLLGNVLCVLAHHFGKEFTPPVQAAAYQKVVAGVANALAHKYH
PENFRLLGNVLVIVLSHHFGKEFTPPVQAAAYQKVVAGVANALAHKYH

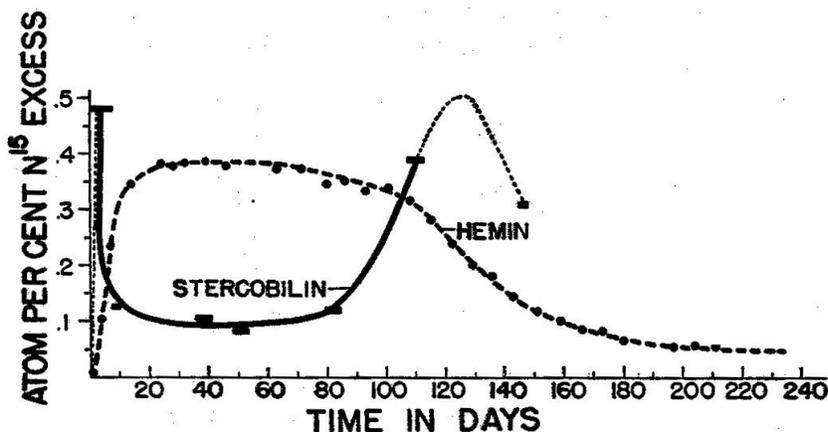
- (3 pts) Indicate with a **forward slash**, “/”, every peptide bond trypsin would hydrolyze.
- (3 pts) Identify those tryptic peptides that have identical sequences in both species by **underlining** them in the human globin sequences?
- (4 pts) Put **boxes** around those rabbit tryptic peptides that would **not** have any ^{14}C in them in Dintzis’ experiment.
- (2 pt) Identify sulfur containing amino acids in each sequence by putting a **circles** around them.
- (2 pt) What is the Fe:S ratio of Human Hemoglobin compared to Rabbit Hemoglobin?

6. (10 points) In parts of West Africa, the β -globin gene is polymorphic in the human populations. That means several alleles coexist at non-negligible frequencies. In a particular population they are β^A , β^S , and β^C at frequencies of 0.82, 0.13 and 0.05 for the normal, sickle cell, and C beta chains, respectively.

A. (5 pts) Draw a modified Punnett Square diagram in which areas represents the proportion of the children born in that population having the various possible genotypes for β -globin genes.

B. (5 pts) On average out of every 10,000 births in this population, how many infants would be born who possess **both** the S and C alleles for the β -globin gene?

7. (16 points) In 1946, Shemin and Rittenberg discovered by accident that glycine was a direct precursor of the heme group of hemoglobin. Shemin consumed a total of 66 g of the ^{15}N -labeled glycine at hourly intervals for almost 3 days. Then he removed samples of his own blood periodically for more than 7 months and had the globin and heme separately analyzed for incorporation of ^{15}N . In related study, he analyzed stercobilin, an excreted product of heme (hemin) breakdown derived from bilirubin, as shown below.



From J. Biol. Chem, 184, 351 (1950)

Fig. 1. ^{15}N concentration in heme and stercobilin of a normal man after the start of feeding ^{15}N -labeled glycine for 2 days.

- A. (6 points) Based on the information in Shemin & Rittenberg (1946) and information you have gained from other sources, construct a model (draw a diagram) of the relationship of heme, globin, bilirubin, and stercobilin to glycine as revealed by pulse-case experiments with ^{15}N from glycine.

B. (5 points) Explain how it is possible for the atom percent ^{15}N excess in stercobilin between 110 and 140 days is greater than it ever was in heme isolated from hemoglobin.

C. (5 points) Shemin and coworkers repeated their study of the incorporation of ^{15}N -labeled glycine into heme on a person who had sickle cell anemia with the results shown at the right. Qualitatively and quantitatively, how does this figure differ from normal? How can these differences be explained?

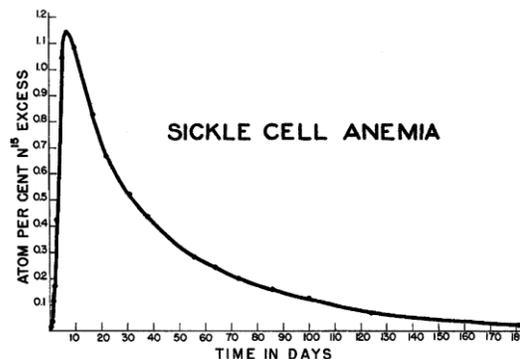


FIG. 4. ^{15}N concentration in heme after feeding ^{15}N -labeled glycine for 2 days

From J. Biol. Chem. 179, 468 (1949)

8. (15 Points) Screening of hemoglobins in blood samples revealed the interesting case of a husband and wife who were each heterozygous for a different hemoglobin variant. Based on the electrophoretic pattern, researchers initially thought the husband was heterozygous for HbC ($\alpha_2\beta_2^{6\text{Glu}\rightarrow\text{Lys}}$) and his wife heterozygous for HbS ($\alpha_2\beta_2^{6\text{Glu}\rightarrow\text{Val}}$). However, when they studied the electrophoretic patterns for hemoglobin in the children of the family, it was apparent that the father's mutation affected the alpha chain and not the beta chain as would be the case for HbC.

A. (10 points) In the space indicated, depict the pattern that would be observed in at least one child that would not be seen if the father were actually heterozygous for HbC.



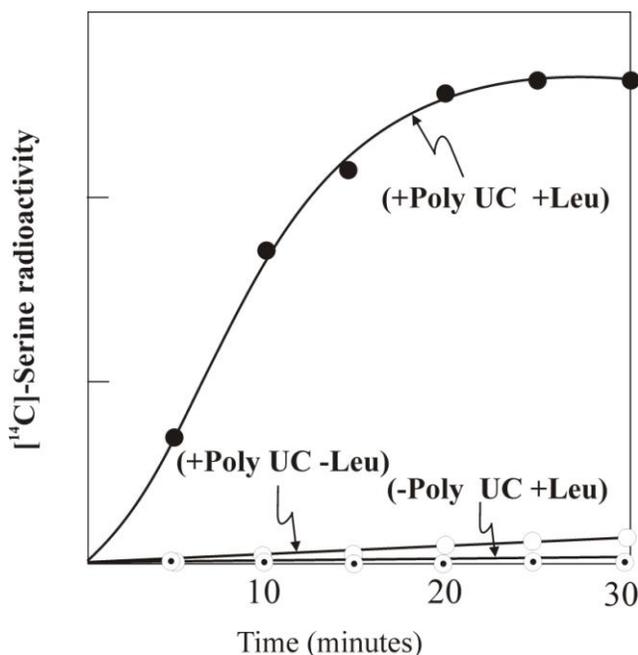
Explain.

B. (5 points) The new variant was purified and digested with trypsin. The resulting two-dimensional peptide map ("fingerprint") showed the loss of one and the gain of two new peptides compared to the alpha chain of HbA. Why would this be expected based on the electrophoretic mobility (of Hb"C")?

9. (10 Points) Dintzis's determination of the direction of protein synthesis in 1961 initiated a decade of remarkable progress in our understanding of protein synthesis and the genetic code as revealed by the 1971 video on *Protein Synthesis* shown in class. The following data come from an experiment in which a synthetic messenger RNA having the repeating ribonucleotide sequence UC_n (poly UC) was used to understand the genetic code. The researchers used three incubation conditions (Table below) and measured the incorporation of radioactivity from the amino acid serine over time as a function of added leucine and poly UC.

Source: H.G. Khorana, in *Nobel Lectures in Molecular Biology 1933-1975* (1977) Elsevier, New York, pp. 303-331.

Reaction additions	Reaction 1	Reaction 2	Reaction 3
<i>E. coli</i> extract (ribosomes, tRNA, enzymes)	+	+	+
[¹⁴ C] Serine, ATP, and GTP	+	+	+
Unlabeled leucine	omit	+	+
Poly UC	+	omit	+



The results of the experiment are shown in the figure to the left. Several important conclusions can be deduced from this experiment.

- A. (5 pt) What is the amino acid sequence of the protein produced?
- B. (5 pt) What are possible triplet codons for leucine based on this experiment?

10. (10 Points) Today, 100 years after Herrick described the first case of sickle cell anemia, we know in detail the molecular structure and properties of HbS and the mutated gene that encodes it. We understand how and under what conditions red blood cells sickle. We know that people with sickle cell trait are resistant to life-threatening attacks of malaria. Yet treatment of patients with sickle cell disease still deals primarily with the symptoms rather than the causes and cures. Basic medical science costing many millions of dollars annually is based on the premise that one must understand a disease in order to treat it or cure it. Does the example of sickle cell anemia invalidate that premise?