

CHEM-342 Introduction to Biochemistry
Final Examination - Group Part
Thursday, 26 May 2005
5:15 – 6:30 PM
H. B. White - Instructor

Group Members

Important - Please read this before you turn the page.

- You must sign your name on this page to receive the group grade. In case of lack of consensus, you may hand in your answer separately from your group for individual grading.
- You may refer to your notes, course reader, handouts, or graded homework assignments. Reference books in the course library may be consulted briefly and returned.
- In CHEM-342, hemoglobin is a vehicle for learning how to learn by asking questions and pursuing answers to those questions. Undoubtedly you have learned a lot about hemoglobin in the process but you also should be developing habits of mind that will enable you to solve problems in other courses and throughout your life. This part of the final examination provides an opportunity for you and the other members of your group to display problem-solving skills as a team. It is extremely unlikely that anyone in your group or in the class has encountered the information on the following page and handout sheet. Your answers should display your collective:
 - breadth of knowledge (not limited to hemoglobin or biochemistry)
 - ability to analyze, make connections, and ask probing questions
 - sense of logic and organization
 - skill at generating models (testable hypotheses)
- This examination should be approached in phases
 - Phase 1** - Individual work for 10 to 15 minutes. Read the questions carefully and write down all of your learning issues and thoughts.
 - Phase 2** - Starting about 5:30 PM, as a group, generate as long a list of learning issues as you can. Organize your learning issues into five major categories and arrange them to display a probing series of connected questions.
 - Phase 3** - Generate a hypothesis provoked by the data and built around one of your learning issue categories. Make a prediction about the results of a test of your hypothesis.
- This examination will be evaluated on the richness of learning issues, the depth of analysis, the presentation of learning issues, and the quality of hypotheses and tests.

1. Among humans, many different hemoglobin variants exist. Most are rare and due to a point mutation. Typically, each differs from HbA by a single amino acid replacement. Some have pathological effects while others are benign or, very occasionally, beneficial in some way. With the passing exception of closely related species, e. g. humans and chimpanzees, hemoglobins differ from one species to another. Over time, the processes of random mutation, genetic drift, and natural selection result in hemoglobin molecules with different amino acid sequences and sometimes with distinctive properties adapted to quite different environments be they bar-headed geese that can fly at 30,000 feet in the thin air over the Himalayan Mountains or, for this question, fish that can swim deep in the ocean.

Many fish have an oxygen-filled swim bladder. By changing the volume of this balloon-like sac, fish can rise, sink, or maintain neutral buoyancy at some depth. Oxygen, extracted from the water by a specially adapted hemoglobin in the gill capillaries is released by a “gas gland” into the swim bladder. Lactic acid produced by the gas gland acidifies the blood and the hemoglobin from these fishes release about half of their bound oxygen. (Compare pH 7.4 and 6.4 on the Hill Plot for tuna hemoglobin.) This dramatic acid-dependent reduction in oxygen affinity, an exaggerated Bohr Effect, is known as the Root Effect and its evolution was the subject of an interesting article in the March 18th issue of *Science* this year.

Hemoglobins that display the Root Effect have a distinctive amino acid composition signature in that they contain about half the number of histidine residues compared to hemoglobins without the Root Effect. For example, as shown on the handout, the hemoglobin from the bluefin tuna displays a Root Effect and has only 10 histidines compared to 19 in humans. Its amino acid sequence is known and its X-ray structure were recently determined.

- a. (15 Points) Based on this information and that provided on the accompanying information sheet, generate learning issues in at least five different categories.
- b. (10 Points) Identify one learning issue that makes a nontrivial experimentally testable hypothesis. What are the predictions of your hypothesis?

Learning Issue Category 1 _____

Learning Issue Category 2 _____

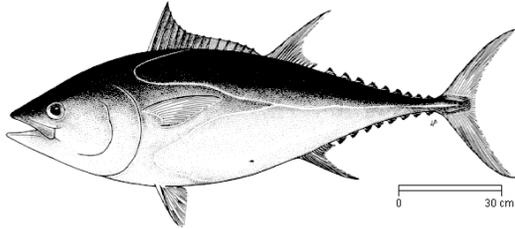
Learning Issue Category 3 _____

Learning Issue Category 4 _____

Learning Issue Category 5 _____

State a testable hypothesis generated in one category of learning issues. What would you need to do test your hypothesis? What are your predictions?

The Bluefin Tuna, *Thunnus thynnus*

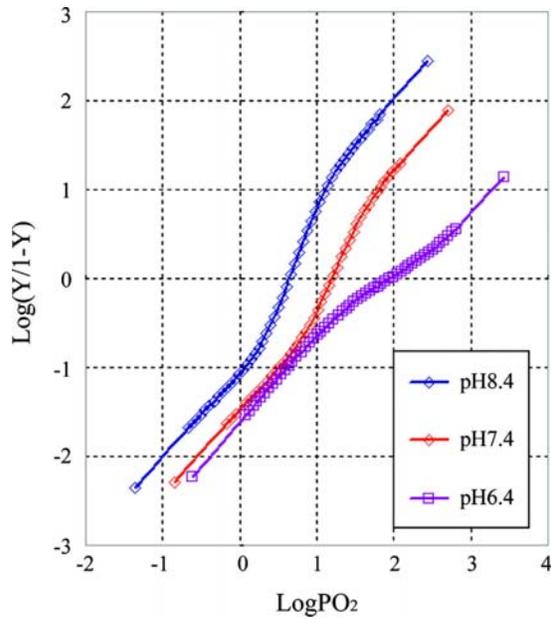


FAO



Researcher holding a swim bladder from a large fish.

Science **307**, 1705 (2005)



Hill Plot of Bluefin tuna, *Thunnus thynnus*, hemoglobin. *JBC* **279**, 28632 (2004)

Amino acid	Human alpha	Human Beta	Tuna alpha	Tuna beta
Gly	7	13	10	10
Ala	21	15	16	17
Val	13	18	12	13
Leu	18	18	15	14
Ile	0	0	5	10
Phe	7	8	6	7
Tyr	3	3	3	6
Trp	1	2	2	2
Cys	1	2	1	3
Met	2	1	5	1
Ser	11	5	13	5
Thr	9	7	8	6
Asn	4	6	1	8
Gln	1	3	1	5
Asp	8	7	11	10
Glu	4	8	3	6
His	10	9	5	5
Lys	11	11	15	8
Arg	3	3	4	6
Pro	7	7	7	4

Alignment of Human and Bluefin Tuna alpha and beta Globins

ALIGN calculates a global alignment of two sequences version 2.0u, Myers and Miller, CABIOS (1989) 4:11-17

Alpha Chain Globins 54.2% identity;

```
HUMAN  VLSPADKTNVKAAWGKVGAHAGEYGAEALERMFLSFPTTKTYF
TUNA  AcTTLSDKDKSTVKALWGKISKSAIDAIGADALGRMLAVYPQTKTYF
```

```
PHF-DLSHGSAQVKGHGKKVADALTNVAHVDDMPNALSALSDLHAHKLRV
SHWPDMSPGSGPVKAHGKVMGGVALAVSKIDDLTTGLGDLSELHAFKMRV
```

```
DPVNFKLLSHCLLVTLAAHLPAEFTPAVHASLTKFLASVSTVLTSKYR
DPSNFKILSHCILVVAKMFPKEFTPDAHVSLDKFLASVALALAERYR
```

Beta Chain Globin 47.6% identity

```
HUMAN  VHLTPEEKSAVTALWGKVNVDVEVGGEALGRLLVVYPWTQRFFES
TUNA   VEWTQQERSIIAGIFANLNYEDIGPKALARCLIVYPWTQRYFGA
```

```
FGDLSTPDAVMGNPKVKAHGKKVLGAFSDGLAHLNLTGTFATLSELHCDK
YGDLSSTPDAIKGNAKIAAHGVKVLHGLDRAVKNMDNINEAYSELSVLHSDK
```

```
LHVDPENFRLLGNVLCVLAHHFGKEFTPPVQAAYQKVVAGVANALAHKYH
LHVDPDNFRILGDCLTVVIAANLGDFTVETQCAFQKFLAVVVFALGRKYH
```

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	