HOW DO FIBERS FORM?

INTRODUCTION

In the short film *The Making of the Fittest: Natural Selection in Humans* you observed normal and sickled red blood cells (RBCs). What causes the RBCs of individuals with the sickle cell allele to become misshapen?

Hemoglobin (Hb) is the oxygen-transporting protein found in the RBCs of almost all vertebrates. Hb makes up approximately 97 percent of the dry weight of an RBC. In humans, the Hb molecule is an assembly of four protein chains: two alpha chains and two beta chains. Each chain folds around a ring-like heme group that contains an iron atom. Oxygen molecules bind to the iron atom, making it possible for a single Hb molecule to carry up to four oxygen atoms at one time.

The genes for the protein chains of Hb show slight variations within different human populations. In fact, the amino acid sequence of Hb is slightly different from one person to the next. The changes seldom affect the ability of Hb to function properly. That is not true in the case of the sickle cell Hb (HbS); see the figure1 below.

Figure 1. Comparison of Wild-Type and Mutant Hemoglobin Beta Chains

Fragment of the Beta Chain of Wild-Type (Normal) Adult Hemoglobin (HbA)

DNA Code

3'-	CAC	GTG	GAC	TGA	GGA	CTT	CTC	-5'

↓

Messenger RNA (mRNA) Code

5'-	GUG	CAC	CUC	ACU	CCU	GAA	GAG	-3'

↓

Amino Acid Sequence

	Val	His	Leu	Thr	Pro	Glu	Glu	

Fragment of the Beta Chain of Sickle Cell (Mutant) Hemoglobin (HbS)

DNA Code

3'-	CAC	GTG	GAC	TGA	GGA	CAT	CTC	-5'

↓

mRNA Code

5'-	GUG	CAC	CUC	ACU	CCU	GUA	GAG	-3'

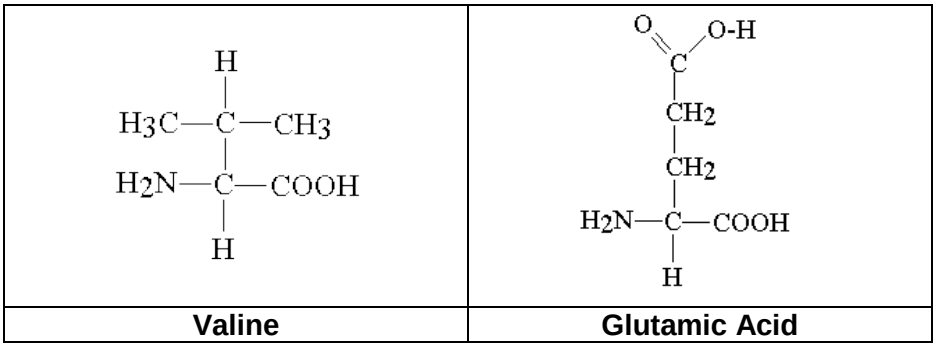
↓

Amino Acid Sequence

	Val	His	Leu	Thr	Pro	Val	Glu	

As you can see, the only difference between the HbA and HbS fragments is in the sixth codon. A point mutation in the DNA results in amino acid number six being different in the two beta Hb protein chains. In HbA, the sixth amino acid in the sequence is glutamic acid (Glu). In sickle cell hemoglobin, it is valine (Val). Individuals with two copies of the mutation (homozygotes) have the disease sickle cell anemia. Individuals with only one copy (heterozygotes) carry the mutated allele for HbS but do not show symptoms of the disease.

Figure 2. The Chemical Structures of Valine and Glutamic Acid.



Valine	Glutamic Acid

Valine is nonpolar (see Figure 2), so it is a molecule that does not have oppositely charged ends, and we describe it as being hydrophobic, which literally means "afraid of water." This is not the case with glutamic acid, which has a negative charge. The change in the charge of an amino acid on the surface of the HbS molecule is what causes HbS to associate with other HbS molecules when in the deoxygenated state.

Here is how this association occurs. When the heme group of an Hb molecule is not carrying any oxygen, which means Hb is in a deoxygenated state, both HbA and HbS take on slightly different three-dimensional shapes than the oxygenated forms. In deoxygenated HbA and HbS, a hydrophobic pocket forms on the surface of the protein. This newly exposed hydrophobic area in one HbS molecule interacts with the mutated region containing the valine in another HbS molecule. As a result, mutated HbS molecules tend to stick to one another and produce stiff fibers inside the RBCs (see figure 3). These fibers cause the RBCs to sickle. Homozygotes will have many sickle-shaped cells when blood is low in oxygen; heterozygotes may also have some sickle-shaped cells, for example, when doing strenuous exercise at high altitudes. For the most part, the RBCs of heterozygotes appear normal. Because HbA does not contain the surface valine, HbA fibers do not form.

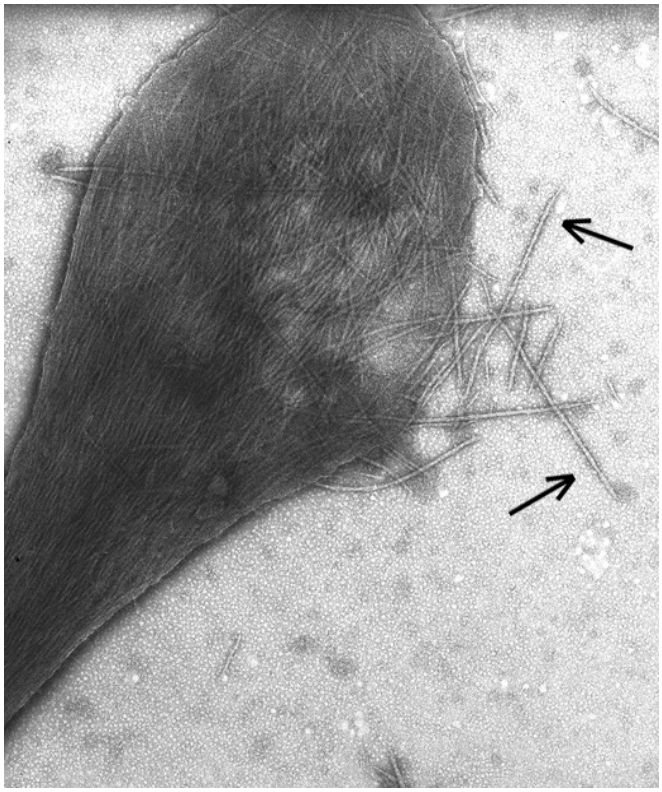
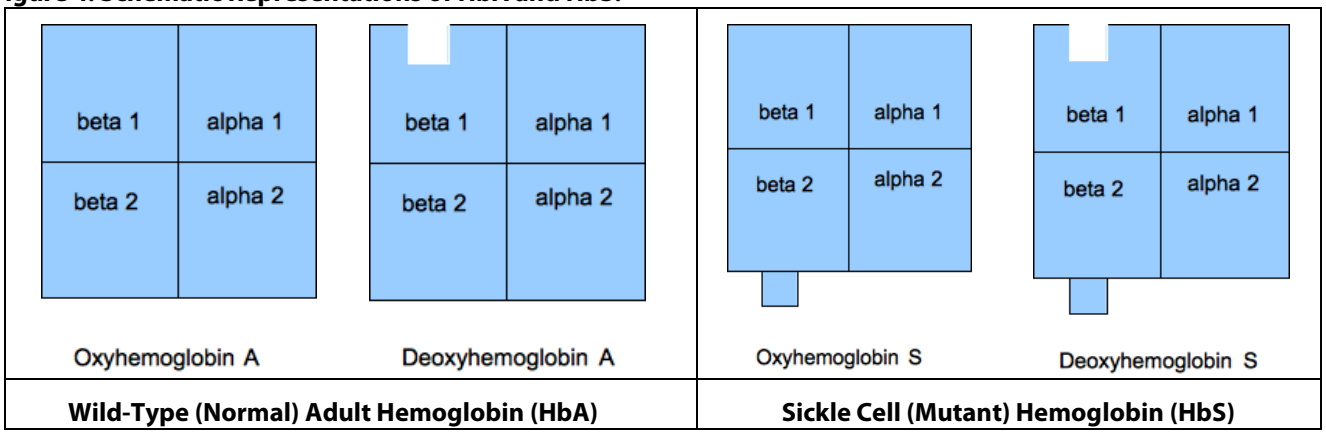


Figure 3. Sickled-shaped Red Blood Cell Filled with Sickle Hemoglobin Fibers. Several fibers (see arrows) are outside the cell. (Image courtesy of William Stokes and Robert Josephs. Based on the work of Thomas E. Wellems, Bridget Carragher, David Bluemke, Stanley Watowich, Leon Gross, Robert Vassar, Michael Potel, and Robert Josephs.)

In figure 4 below, one-quarter of each square in each of the four Hb models represents one protein chain. Remember that four protein chains (two alpha and two beta) make up a single Hb molecule. The mutation in the sixth codon of the beta chain is represented in the two HbS molecules by a square protrusion. The square notches in the beta chains of both the HbA and HbS deoxyhemoglobin molecules represent the hydrophobic pocket that forms when oxygen is released.

Although both beta chains in an HbS molecule have the valine mutation (or the square protrusion) and both beta chains in deoxygenated HbS and HbA molecules have the pocket, only one beta chain from any one molecule will act as the donor for the interaction, and the other beta chain will act as the acceptor. As a result, we depict only one beta chain per molecule as having the protrusion and the other as having the pocket. Of course in real life, proteins look nothing like this. They are complex, three-dimensional structures with all kinds of nooks and crannies.

Figure 4. Schematic Representations of HbA and HbS.



Source: The drawings were adapted from Dr. Ishita Mukerji. "Fiber Formation." About Sickle Cell Disease. http://www.sicklecellinfo.net/fiber_formation.htm.

MATERIALS

- scissors
- tape
- copies of the four types of Hb molecules
- eight mosquito netting circles, each with an 8-centimeter diameter
- four sheets of blank copy paper

PROCEDURE

1. Organize the mosquito netting circles into four groups of two each. The circles represent the cell membranes of the RBCs. (In real life, RBCs have a diameter of 6–8 micrometers, or 0.0006–0.0008 centimeters.)

2. Cut out the models of the four types of Hb molecules (see pages 7–8). Keep them sorted by type: oxyhemoglobin A and deoxyhemoglobin A, oxyhemoglobin S and deoxyhemoglobin S.

3. Model the RBC of oxygenated normal hemoglobin by doing the following tasks.

a. Oxyhemoglobin A represents normal hemoglobin in an oxygen-rich environment. Because oxyhemoglobin A molecules have no notches or protrusions, which can act as acceptors or donors in chemical bonding, they float freely inside the fluid environment of the RBC.

b. Place one mosquito netting circle in the center of a blank sheet of copy paper on the desk in front of you.

c. Arrange your pile of oxyhemoglobin A on top of the mosquito netting circle to simulate the behavior of hemoglobin inside the RBC.

d. Do all the hemoglobin molecules fit within the 8-centimeter circle? (yes or no)

e. If yes, continue to Step 3f. If no, stretch the circle on the desk so that it conforms to the shape that the hemoglobin molecules make.

f. To complete the model, place a second circle over the top of the first circle and stretch it to the same shape. If you don't have to stretch it, leave it as is. Trace around the RBC to record its shape.

Is the shape of the model RBC containing oxyhemoglobin A normal or sickled? _____

4. Now model the RBC of deoxygenated normal hemoglobin.

a. Deoxyhemoglobin A represents normal hemoglobin in an oxygen-poor environment. A lack of oxygen causes a structural change in the hemoglobin protein. A hydrophobic pocket on the beta chain appears, which can act as an acceptor in a chemical bond. We depict this in the paper model as a notch. However, because there is no donor to the chemical bond, even with this hydrophobic pocket present, normal hemoglobin A will be free-floating inside the fluid environment of the RBC.

b. Repeat Steps 3b–3f, basing your work on the information above.

Is the shape of the model RBC containing deoxyhemoglobin A normal or sickled? _____

5. Next, model the RBC of oxygenated sickle cell (mutant) hemoglobin by doing the following tasks.

a. Oxyhemoglobin S represents the mutant sickle cell hemoglobin in an oxygen-rich environment. Remember that the mutation replaces a negatively charged amino acid (glutamic acid) with a nonpolar, hydrophobic amino acid (valine). This mutation is on the surface of the hemoglobin protein, and we depict it with the protrusion on the paper model. The protrusion acts as a donor to a chemical bond. Because this protrusion does not have an acceptor, in an oxygen-rich environment, the mutant hemoglobin remains free-floating in the fluid environment of the RBC.

b. Repeat Steps 3b–3f, basing your work on the information above.

Is the shape of the model RBC containing oxyhemoglobin S normal or sickled? _____

6. Finally, model the RBC of deoxygenated sickle cell (mutant) hemoglobin.

a. Deoxyhemoglobin S represents the mutant sickle cell hemoglobin in an oxygen-poor environment. An oxygen-poor environment causes the hydrophobic pocket (notch) to appear. Also, the mutated sickle cell hemoglobin has the hydrophobic valine protruding from the surface of the hemoglobin protein. Due to the watery environment in the RBC, hydrophobic regions tend to associate with each other in order to get away from the water. A hydrophobic pocket can act as an acceptor and the hydrophobic protrusion can act as a donor in a chemical bond. This causes the hemoglobin proteins to associate with one another to form long fibers.

b. Repeat Steps 3b–3f, basing your work on the information above.

Is the shape of the model RBC containing oxyhemoglobin S normal or sickled? _____

QUESTIONS

1. Explain why fibers do not form in either oxygen-rich or oxygen-poor environments in individuals who have HbA molecules.

2. Explain why fibers do not form in oxygen-rich conditions in individuals who have HbS molecules.

3. Explain why fibers do form in oxygen-poor conditions in individuals who have HbS molecules.

4. Explain how the formation of fibers results in the sickling of RBCs.

5. Read the passage below and answer Questions 5 and 6.

During the short film, you observed that the malaria parasite spends part of its life cycle inside RBCs and that individuals heterozygous for sickle cell disease are somewhat resistant to malaria. Although heterozygous individuals do not have sickle cell disease, some sickling of their RBCs occurs when oxygen levels are low. There are many reasons why oxygen levels can decrease in the blood. One of them is that the malaria parasite reduces the amount of oxygen present in the RBCs as it carries out its metabolism. Thus, infection with malaria can increase sickling of RBCs that contain HbS.

When oxygen is low, sickling occurs because the membranes of RBCs are very elastic. Normally, this elasticity is a positive characteristic for blood cells. It allows RBCs that are 8 micrometers in diameter to easily pass through capillaries half that size. Due to their elasticity, RBCs change shape as they squeeze through capillaries and then resume their normal shape when they enter larger vessels. Sickle-shaped cells cannot squeeze through capillaries and can thus block blood flow, causing symptoms of sickle cell disease. In addition, the body senses that sickle-shaped RBCs are misshapen and marks them for destruction. Some are attacked and destroyed by phagocytes. Others are removed from circulation and destroyed in the spleen or the liver.

When the malaria parasite infects RBCs, it sends proteins called adhesion molecules to the surface of the RBCs. These adhesion molecules cause the RBCs to stick to the walls of the capillaries. One result is that not all of the infected cells get to the spleen or liver for removal. Many remain in the capillaries, where they block circulation to vital organs.

a. Explain why heterozygous individuals might have less severe malaria infections.

b. Explain why the same responses that provide some protection to a heterozygous individual would be very harmful in an individual who is homozygous for sickle cell hemoglobin.

6. Now that you have examined the mechanism by which fibers form and cause cells to sickle, explain how the formation of sickled cells leads to anemia.

AUTHOR
Mary Colvard, Cobleskill-Richmondville High School (retired), New York

Hemoglobin Models

Wild-Type (Normal) Adult Hemoglobin (HbA)

beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1
beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2
Oxyhemoglobin A		Oxyhemoglobin A		Oxyhemoglobin A		Oxyhemoglobin A		Oxyhemoglobin A	
beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1
beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2
Oxyhemoglobin A		Oxyhemoglobin A		Oxyhemoglobin A		Oxyhemoglobin A		Oxyhemoglobin A	

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beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1
beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2
Deoxyhemoglobin A		Deoxyhemoglobin A		Deoxyhemoglobin A		Deoxyhemoglobin A		Deoxyhemoglobin A	
beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1	beta 1	alpha 1
beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2	beta 2	alpha 2
Deoxyhemoglobin A		Deoxyhemoglobin A		Deoxyhemoglobin A		Deoxyhemoglobin A		Deoxyhemoglobin A	

Hemoglobin Models

Sickle Cell (Mutant) Hemoglobin (HbS)