1. Insects lack red blood cells. Instead, an oxygen carrier/protein very similar to hemoglobin is secreted directly into insect blood. Briefly hypothesize how the synthesis of this protein differs from that of its vertebrate relative and describe one direct test of your hypothesis and its possible results.

A. What does the question ask?

> *Insects lack red blood cells. Instead, an oxygen carrier/protein very similar to hemoglobin is secreted directly into insect blood . . .*

The first two sentences of the question asks you to think about the synthesis of two similar proteins, one free in the blood (insects) and one contained within a red blood cell (vertebrate). While implicitly stated in the question, basic knowledge of red blood cell structure and function (from lab or lecture) is necessary to know that vertebrate hemoglobin is contained in erythrocytes:

> *Briefly hypothesize how the synthesis of this protein differs from that of its vertebrate relatives . . .*

The question then asks you to trace the path of the insect oxygen carrier/protein during its synthesis and compare the path to hemoglobin synthesis (not secreted).

> *. . .and describe one direct test of your hypothesis and its possible results.*

Finally, you are asked to design an experiment to test your hypothetical pathway. Furthermore, the question asks to discuss possible results. Thus, answering this question **completely** requires that:

1. You recall how secreted and non-secreted proteins are synthesized,
2. You formulate a hypothesis with regards to the insect oxygen carrier, a secreted protein,
3. You briefly describe a test, **and**
4. You describe possible results of the test.

All four parts are crucial to an excellent answer.

Proposing an experiment is not enough; you must discuss possible results! As long as the proposed experiment is logically based on a rational answer to the previous parts of the question, credit will be awarded even though your "facts" may be partially or completely wrong. Several experiments based on readings, lecture and lab notes, as well as your own logical creativity are possible.

2. What question is NOT being asked?

No functional information about hemoglobin or oxygen carrier/protein is required to answer the question. Also it might be tempting to include information about the osmotic behavior of red blood cells because one lab was dedicated to the subject, but such information is not relevant to the question and should be omitted. **You may be penalized for including extraneous material but more importantly doing so wastes your time.**

3. What's ambiguous about the question?

If anything doesn't make sense to you, ask the professor specific pointed questions. Don't say, "I don't understand the question." instead ask "What do you mean by __________?" or "Could you clarify __________?"

4. Now answer the question before proceeding further.

QUESTION: **Insects lack red blood cells. Instead, an oxygen carrier/protein very similar to hemoglobin is secreted directly into insect blood. Briefly hypothesize how the synthesis of this protein differs from its vertebrate relative and describe one direct test of your hypothesis and its possible results.**

Sample Responses: Answer #1

Overview: He begins the question by reminding himself that hemoglobin is within red blood cells while insect protein is secreted. Based on this fact, he traces the path of synthesis for a secreted peptide and compares it with hemoglobin's synthesis. He concludes that the oxygen carrier is secreted because it has a signal sequence (and possible targeting sequences) which hemoglobin lacks. He proposed to test for the presence of a signal sequence on the oxygen carrier by analyzing mRNA. The oxygen carrier is expected to have a hydrophobid end.

ANSWER

Because the insect protein is secreted directly into the blood, it will be co-translationally imported in the ER and targeted for the secretory vesicles. Hemoglobin, on the other hand, is a product of red bloods cells, and remains within these cells. Therefore, the insect protein will, when it is first translated, begin with a hydrophobic signal sequence to allow it to enter the ER. (It may also have a second signal added to it in the ER or Golgi to target it to the secretory vesicles.) We might be able to test the idea that the insect protein will begin with a signal sequence while the hemoglobin will not by isolating the genes for each in vitro and analyzing their products. We expect the oxygen carriers produced by insects to have a hydrophobic end and hemoglobin not to.

COMMENTARY

The synthesis pathways are clearly and correctly traced in the first paragraph. The alternating structure of the 1st 2 sentences is effective especially for answering compare/contrast questions.

The hypothesis is basically that the insect carrier protein is secreted because it contains a signal sequence. Hemoglobin does not contain such a sequence, so he designed an experiment to detect the sequence.

A good test, but unfortunately he doesn't specifically state how he intends to do so experimentally. He could have referred to specific experimental techniques learned in lecture. In a "brief" answer it is not necessary to provide technical details; nevertheless, more information is required than provided here.

The results he would expect to generate from the experiment are not well described. The results need to be more explicitly related to the hypothesis: 1) hydrophobic end=signal sequence, 2) signal sequence=secretion and 3) secretion=protein directly in the blood.

A Second Example:

Overview: First, the student concludes the syntheses of hemoglobin and the insect oxygen carrier must be different because the fates of the two proteins are different. Then, she proceeds to detail how the oxygen carrier is synthesized, processed, and secreted using text and diagrams. As a logical extension, she decides to experimentally monitor the synthetic pathway of the two proteins by a pulse-chase experiment. The results are presented in a graph and conclusions are drawn from the graphs to explain the differences between the two proteins.

ANSWER

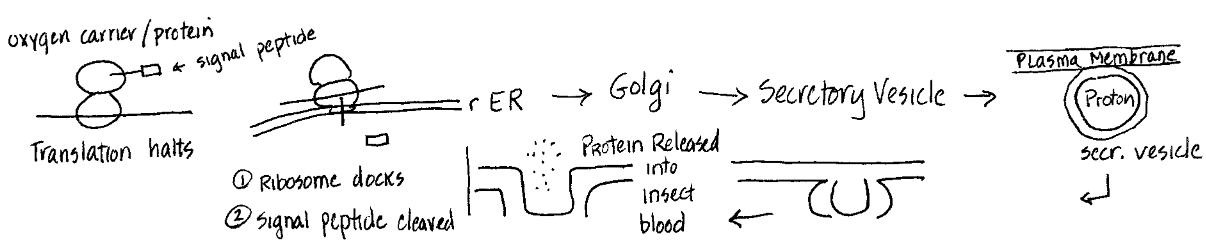
Hemoglobin in RBC is synthesized to stay inside the RBC while the oxygen carrier/protein in insects is synthesized to be exported. As a result, processes of synthesis is different. The hemoglobin is synthesized in the cytoplasm and stays in cytoplasm therefore it lacks a signal peptide. The oxygen carrier/protein will be exported so it will have a signal protein that will associate with a stop particle that will halt synthesis until it comes in contact with rER docking site and translation will resume. The signal peptide will be cleaved with signal peptidase. Then the protein will be brought to the cis-side of the Golgi via transitory vesicles where it will be further processed. The protein probably binds to a receptor side on the trans side of the Golgi and a secretory vesicle is formed with the protein inside. The secretory vesicle moves towards the plasma membrane where it fuses with the membrane, the protein is released into the insect blood and the secretory vesicle membrane becomes part of the plasma membrane. Diagrams:

COMMENTARY

The answer is laid out effectively by starting with general knowledge. In doing so, the reader is told what to expect from the rest of the answer; a more detailed comparison of hemoglobin and oxygen carrier pathways.

She traces the pathway of the oxygen carrier protein synthesis from the rER-->Golgi-->secretory-->plasma membrane-->insect blood. Some of the details are fuzzy – e.g., "stop particle" instead of SRP, and it's not clear what "further processed" means. Otherwise, a **Very Good** answer!

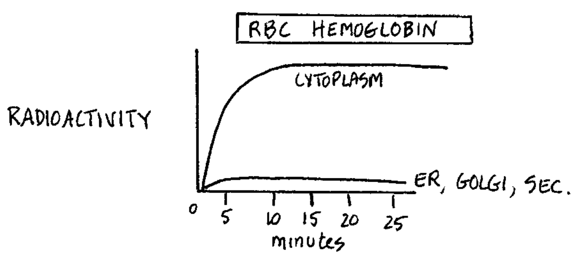
Note how helpful, but how simple, a diagram, is for organizing and expressing thoughts.

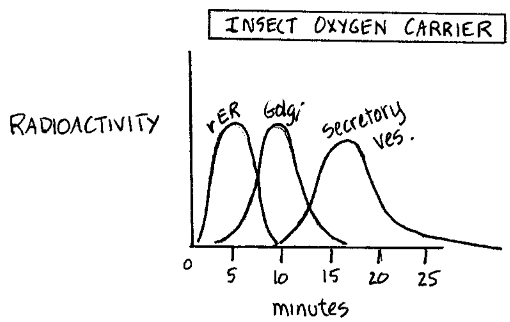


Continuing Example 2:

To observe the movement of the hemoglobin and oxygen carrier/ protein, one could perform a "pulse-chase experiment." Radioactively labeled nucleotides would be added for five minutes and then cells would be incubated with non-labeled nucleotides for 30 minutes and measure where the radioactivity is at certain times. The result probably would show:

An excellent test for this hypothesis, but one requiring radioactive amino acids and not nucleotides!





Graphs are effective ways of communicating data, and in this example they are especially effective for comparing the different pathways.

The exact shapes of the curves are inaccurate, for idiosyncratic reasons beyond the scope of BI250.

More importantly, the data are interpreted correctly and related to the presence of a signal sequence and the fates of newly synthesized proteins, either inside or outside the cell. Note also that what is being tested -- the pathway -- is quite different than what was tested in the first Example. Given the contexts, both are reasonable tests and equally correct answers!

Therefore, hemoglobin stays inside the cytoplasm of the cell, and has no signal sequence. The insect oxygen carrier/protein goes through the rER, Golgi, secretory vesicles and is exported and has a signal peptide that allows it into the rER.

An **Excellent** answer!

Now that you've answered the question and examined two responses in detail, review the question and critique the following 2 responses yourself in the space provided on the left.

ANSWER #3

Because the insects' oxygen carrier/protein is a secretory protein, its synthesis will initially differ in that it will produce a hydrophobic signal sequence. This sequence will then be bound by SRP (signal recognition particle) until the ribosome migrates to the rER. Docking protein in the rER membrane will then recognize SRT which in turn activates TRAM, forming a channel in the rER membrane through which the nascent polypeptide may then pass. The hemoglobin of vertebrates, by contrast, will likely be synthesized in the cytoplasm and contain no such signal sequence for import into the rER because it is a cytoplasmic protein.

A test of this hypothesis might be to isolate the gene for the insect oxygen carrier and delete the portion which codes for the signal sequence. This would presumably inhibit the entry of the polypeptide into the rER and thus it would not be secreted.

COMMENTARY

Your critique might usefully lead you to edit the example, making it more accurate in the process. Use the space below for your version –

ANSWER #4

COMMENTARY

An obvious difference is that the insect protein is secreted while hemoglobin is retained within the red blood cell. Thus, the protein (insect) at one point probably had a signal sequence on the amino end that enabled the protein to enter the lumin of the rER. Hemoglobin lacked this type of signal sequence and thus moved into the cytoplasm rather than into the rER.

Once this insect protein went into the rER, it possibly went through some modifications such as losing its signal sequence and/or glycosylation. Hemoglobin had no signal sequence to lose and would not go through glycosylation. Modification such as sulfur bridges may occur with both. In fact, it does with hemoglobin as it gains its quaternary structure. The insect may lack quaternary structure, we cannot tell. However, any tertiary or quaternary structure will take place here.

The hemoglobin does not leave the cytoplasm but the insect protein moves on through vesicles to the Golgi. Here glycosylation will finish up as will other modifications. From here it moves to secretory vesicles and is secreted. The hemoglobin remains within the RBC.

A way to test if the difference of location of modification does exist between the proteins, one could get some antibodies to the protein and inject them into appropriate cells. These antibodies would cling on to the protein thus locating them. Most probably, the insect protein would be found in the rER, the Golgi, or vesicles going toward the Golgi. Secretory vesicles is another possibility. Hemoglobin will be found in the cytoplasm. If these results were found, the difference exists.

If one wanted to get more specific (i.e. test for glycosylation) one could take both proteins and stain them with dyes that stain positive for glycoproteins. It is possible that the insect protein may contain oligosaccharides. It is very doubtful that hemoglobin would.