ANSWERS
Supplementary Problem Set: Myoglobin and Hemoglobin
Reminder: Work assigned problems in Chapter 7

1. **Where is myoglobin found and what is its biological function? Answer the same question for hemoglobin.**

   Myoglobin in found in skeletal and heart muscle and is a short-term storage protein for oxygen as the oxygen diffuses from the blood stream into the mitochondria and into parts of the cell where oxygen is required for metabolic processes. Myoglobin molecules bind and release oxygen rapidly aiding their transport of oxygen within the cell.

   Hemoglobin is found in red blood cells and transports oxygen from the lungs to all tissues in the organism.

2. **Derive the equation for the proportion of myoglobin that is bound to oxygen (1) using the notation MbO_2 and Mb. Substitute for Mb in terms of MbO_2 and show *(derive)* the alternative form of the equation with the notation $[O_2]$ and P_{50}.** (See lecture notes)

3. **Repeat #2 for hemoglobin.** (See lecture notes)

4. **Choose one equation from #2 or #3 and show the derivation for the Hill equation. Indicate what form this equation has (liner, hyperbolic, sigmoidal) and sketch the Hill plot for the equation you choose labeling the axes properly.** (See lecture notes)

5. **What is the numerical value for n in the equation for 1 for myoglobin and how is this value determined from the Hill plot? What does this value of n represent for myoglobin?**

   The numerical value for n in the 1 equation for myoglobin is 1.0. This value is determined from the slope of the hill plot (log 1 versus log pO_2). The fact that the Hill plot is linear with slope 1.0 is interpreted to mean that only one O_2 binds to myoglobin (n = 1.0) and that there is one value for P_{50} (K_d). For myoglobin, the number of sites is only one because there is only one heme in myoglobin!

6. **At low pO_2 and at high pO_2, the value of n in the Hill equation is 1.0, but at intermediate values of pO_2, the value is ~ 3.0. What property of hemoglobin accounts for the change in n going from low pO_2 to intermediate pO_2 ~~to high pO_2~~?**

   Hill equation for Hb: $\log (\text{bound/free}) = n \log(pO_2) - \log (Kd\ (=1/P_{50}))$

   The value of n changes from 1.0 to 3.0 in going from the low pO_2 to intermediate pO_2 because after the first O_2 is bound, the affinity of Hb for additional O_2 increases (P_{50} increases, K_d ($=1/P_{50}$) decreases) so that the y-intercept has to change as a reflection of the increase in affinity of the other heme sites.

   Additional perspective:

   Hemoglobin is a tetramer having four binding sites for oxygen. The affinity of each heme for O_2 changes (increases) upon the binding of (or with the presence of) O_2 at other heme sites. This means that the value of P_{50} increases (K_d decreases) as successive O_2 are bound to the hemes. At low pO_2, it is likely that only one site per hemoglobin tetramer (on the average) is bound to O_2 and each of these sites has an identical low affinity for O_2, in other words, the same low P_{50}. This situation gives rise to the portion of the Hill plot where the value of n = 1.0 and a y-intercept showing a lower P_{50} value.

   At higher values of pO_2, three sites per hemoglobin (on the average) will be bound to O_2 so that when the fourth O_2 binds, it binds to a high affinity site on each tetramer where the molecules of Hb have the same affinity for O_2, or same high P_{50} value. This situation also gives a Hill plot with slope of 1.0 but with a higher y-intercept giving a higher P_{50} value.

   In the intermediate pO_2 range, there are mixtures of hemoglobin tetramers with 1, 2, 3, and 4 O_2 molecules bound so that the measured value of the slope (n) represents a transitional slope between the low affinity sites (with low P_{50}) and the high affinity sites (with low P_{50}). Since the Hill plot is derived from an approximate (simplified) equation for binding of O_2 to hemoglobin, the interpretation cannot be exact.

   Note that the value of n for the Hill plot for hemoglobin never reaches 4 as implied by the simplified chemical reaction (and its accompanying equations for 1 and the Hill equation) so that the simplified chemical reaction (and its accompanying Hill equation and plot) are not an exact description of the binding to O_2 to hemoglobin! See #7.

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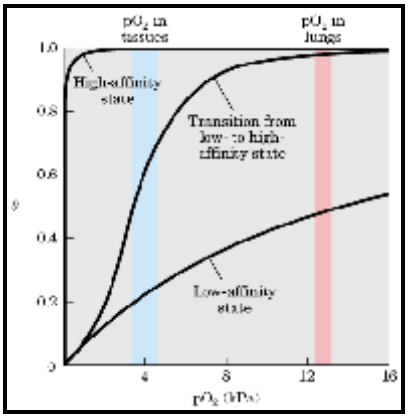
7. How does the two state model for binding of O_2 to hemoglobin explain the sigmoidal dependence of the binding of O_2 to this protein?

The two-state model for the binding of O_2 to hemoglobin explains the sigmoidal dependence of the binding plot because it accounts for the low affinity of Hb for O_2 at low pO_2 where the T-state, or deoxy state is the predominant form as well as the transition to the high affinity for O_2 at high pO_2 where the R-state, or oxy state, is the predominant form.



Additional perspective:

If it were the only form present, the T-state would give a "low-lying" hyperbolic plot; similarly if it were the only form present, the R-state would give a "high-lying" hyperbolic plot (see plot at right). Because it includes the transition between the low affinity state (T-state) and the high affinity state (R-state), the two-state model explains the sigmoidal appearance of the binding plot!

A sigmoidal plot can actually be derived from the proper mathematical weighting of the concentration of these two forms as the pO_2 changes, as well as a proper assignment of two different values of P_{50} that would be assigned to them. Needless to say, your text book does not present this mathematical analysis!

8. Describe the changes that happen to the following groups or interactions in hemoglobin when oxygen binds to deoxyhemoglobin.

a. Salt bridge between groups on the H and F-helices: H-asp-CO_2^{-}·····$^{+}$HN<his-F

This salt bridge breaks!

b. Position of the F-helix with respect to the heme plane

The F-helix moves toward the heme plane

c. Proximal histidine with respect to the heme plane

The proximal HIS moves toward the heme plane

d. Position of Fe^{2+} with respect to the heme plane

The Fe^{2+} moves toward the heme plane

e. Number of salt bridges in the $\alpha_1\beta_2$ and $\alpha_2\beta_1$ interfaces

The number of salt bridges between the α and β chains is reduced upon the binding of O_2.

f. Binding of BPG in a cavity formed by positively charged groups on the β_1 and β_2 chains.

BPG dissociates from the cavity

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9. Explain why the changes you listed in 8a occur at the salt bridge between groups on the H and F-helices upon the binding of O_2 to deoxyhemoglobin.

When O_2 binds, the Fe^{2+} moves toward the heme plane the proximal his to move and hence the F helix to move; this movement moves the HIS farther away from the ASP so that the salt bridge "breaks", the histidine pK_a drops, and the H^+ is dissociates.

10. Describe the Bohr effect and how it is important for the function of hemoglobin. Explain why myoglobin does not exhibit a Bohr effect.

The Bohr effect is the drop in the saturation of hemoglobin that occurs with a decrease in pH and the binding of CO_2 to the N-terminal $-NH_2$ groups. This effect is important in for the function of hemoglobin because it allows hemoglobin to release O_2 to the tissues that need it and which are releasing H^+ and CO_2 as a result of metabolizing fuels. This drop in saturation occurs because the binding of the H^+ and CO_2 result in conformational changes in the three dimensional structure of hemoglobin that move the Fe^{2+} away from the heme plane weakening the binding to O_2.

Myoglobin does not exhibit a Bohr effect because it does not have quaternary structure to regulate the degree of saturation by O_2. Myoglobin alternatively binds and releases O_2 as the O_2 makes its way from the blood stream into cells and on into the mitochondria.

11. What is the function of 2,3-bisphosphoglycerate (BPG)? Why do red blood cells have large amounts of BPG?

BPG stabilizes the deoxy form of hemoglobin. When it binds in a cavity made by the β chains, BPG shifts the equilibria from the oxyhemoglobin to the deoxyhemoglobin forms, thereby promoting the loss of O_2.

12. Why are myoglobin and hemoglobin highly colored red?

Myoglobin and hemoglobin absorb green to yellow light in the 500 nm to 600 nm region of the spectrum. Red light is transmitted through solutions containing these proteins so the solutions appear to be red in color!

13. Describe why the genetic change of a glu (Hb-A) for a val (Hb-S) results in the condition called "sickle cell anemia".

Go to http://gingi.uchicago.edu/sc-tour1.html for animated gif images of fibers formed from aggregated Hb molecules.

The following was adapted from http://peptide.ncsa.uiuc.edu/tutorials_current/Sickle_Cell_Anemia/

Long fibers of deoxyhemoglobin molecules form since the mutated valine-6 residues lead to the aggregation of the Hb molecules. The aggregation is driven by the increase in entropy of the surrounding water (valine side chain is hydrophobic) so that Hb molecules keep aggregating as the disorder continues to increase.

As they form, the fibers cause the shape of the red blood cell to become sickle-shaped. The long fibers lead to distortion of the cell membrane, causing the characteristic "sickle" shape of the red blood cells associated with the disease. The sickled cells can no longer move normally through the blood vessels, so normal delivery of oxygen to the body is interrupted. These distorted cells are removed from the blood stream by the pancreas leading to anemia.